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(54) **COMPOSITIONS AND METHODS FOR
PRODUCING FERMENTABLE
CARBOHYDRATES**

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(58) **Field of Classification Search**
None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,902,622	A	2/1990	Nakai et al.
5,750,875	A	5/1998	Stalker et al.
5,786,140	A	7/1998	Mattes et al.
6,127,603	A	10/2000	Nicholas
6,235,971	B1	5/2001	Barry et al.
6,664,444	B1	12/2003	Koops et al.
7,208,307	B2	4/2007	Mattes et al.
7,235,712	B1	6/2007	Zhang et al.
7,655,836	B2*	2/2010	Birch et al. 800/284
2007/0077569	A1	4/2007	Birch et al.
2007/0240240	A1	10/2007	Birch et al.

FOREIGN PATENT DOCUMENTS

JP	63-129990	A	6/1988
WO	WO 89/12386	A1	12/1989
WO	WO 91/19808	A1	12/1991
WO	WO 95/13389	A1	5/1995

WO	WO 01/59131	A2	8/2001
WO	WO 02/18603	A1	3/2002
WO	WO 02/27003	A1	4/2002
WO	WO 03/018766	A2	3/2003
WO	WO 2004/099403	A1	11/2004
WO	WO 2005/096804	A2	10/2005

OTHER PUBLICATIONS

Börnke et al, 2001, J. Bacteriol., 183:2425-2430.*
Birch et al, 2007, Plant Biotech., 5:109-117.*
Bornke, F., et al., "Cloning and Characterization of the Gene Cluster for Palatinose Metabolism from Phytopathogenic Bacterium *Erwinia rhapontici*," *J. Bacteriology*, Apr. 2001, pp. 2425-2430, vol. 183, No. 8.
Cheetham, P.S.J., "The Extraction and Mechanism of a Novel Isomaltulose-synthesizing Enzyme from *Erwinia rhapontici*," *Biochem. J.* (1984), pp. 213-220, vol. 220.
Galvez-Mariscal, A., and A. Lopez-Munguia, "Production and Characterization of a Dextranase from an Isolated *Paecilomyces lilacinus* Strain," *Appl. Microbiol Biotechnol.*, 1992, pp. 327-331, vol. 36.
Jimenez, E.F., "The Dextranase Along Sugar-making Industry," *Biotechnologia Aplicada*, 2005, pp. 20-27, vol. 22.
Loreti, E., et al., "Glucose and Disaccharide-Sending Mechanisms Modulate the Expression of α -amylase in Barley Embryos," *Plant Physiology*, Jul. 2000, pp. 938-948.
Ohta, K. et. Al., "Production of High Concentrations of Ethanol from Inulin by Simultaneous Saccharification and Fermentation Using *Aspergillus niger* and *Saccharomyces cerevisiae*," *Applied and Environmental Microbiology*, Mar. 1993, pp. 729-733, vol. 59, No. 3.
Salvucci, M.E., Distinct Sucrose Isomerases Catalyze Trehalulose Synthesis in Whiteflies, *Besmisia argentifolii*, and *Erwinia rhapontici*, *Comparative Biochemistry and Physiology*, 2003, pp. 385-395, Part B, No. 135.
Watanabe, K., et al., "Proline Residues Responsible for Thermostability Occur with High Frequency in the Loop Regions of an Extremely Thermostable Oligo-1,6-glucosidase from *Bacillus thermoglucosidasius* KP1006," *J. Biol. Chem.*, Dec. 25, 1991, pp. 24287-24294, vol. 266, No. 36.

(Continued)

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(57) **ABSTRACT**

Provided herein are methods for producing fermentable sugar obtained from a plant tissue. The methods include providing transgenic plant material comprising one or more locked carbohydrates and contacting plant material with an enzyme capable of converting the locked carbohydrate into a fermentable sugar. The methods are useful for providing sugar or sugar pre-cursors for several industrial purposes including ethanol production. The invention also encompasses plants and plant parts that produce a lock enzyme to yield a locked carbohydrate, with the consequence of accumulating the locked carbohydrate in the plant. The invention also encompasses providing a key enzyme able to convert locked carbohydrates to fermentable sugars. Key enzymes can be provided by transgenic plants or plant parts, transgenic microbes, transgenic yeast, microbes or yeast.

22 Claims, No Drawings

(56)

References Cited

OTHER PUBLICATIONS

Wu, L., and R.G. Birch, "Doubled Sugar Content in Sugarcane Plants Modified to Produce a Sucrose Isomer," *Plant Biotechnology Journal*, 2007, pp. 109-117. vol. 5.

Zhang, D., et al., "Isomaltulose Synthase from *Klebsiella* sp. Strain LX3: Gene Cloning and Characterization and Engineering of

Thermostability," *Applied and Environmental Microbiology*, Jun. 2002, pp. 2676-2682, vol. 68, No. 6.

Form PCT/ISA/220 Transmittal of International Search Report dated Sep. 17, 2009, for parent PCT Application No. PCT/US2009/046968.

* cited by examiner

COMPOSITIONS AND METHODS FOR PRODUCING FERMENTABLE CARBOHYDRATES

RELATED APPLICATIONS

This application is a national phase application claiming the benefit of priority under 35 U.S.C. §371 to Patent Convention Treaty (PCT) International Application Serial No. PCT/US2009/04698 having an international filing date of Jun. 11, 2009 (published as WO 2009/152285, on Dec. 17, 2009), which claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Application Ser. No. 61/060,789 filed Jun. 11, 2006. The aforementioned applications are explicitly incorporated herein by reference in their entirety and for all purposes.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The official copy of the sequence listing is submitted concurrently with the specification as a text file via EFS-Web, in compliance with the American Standard Code for Information Interchange (ASCII), with a file name of "71825USPSP2 sequence listing.txt, created Jun. 10, 2009, and a size of 313 KB. The sequence listing filed via EFS-Web is part of the specification and is hereby incorporated in its entirety by reference herein.

FIELD OF THE INVENTION

This invention relates to plant molecular biology, particularly to methods and compositions for improving plants for obtaining commercially desirable harvested plant material, particularly for ethanol production.

BACKGROUND OF THE INVENTION

Plant biomass is comprised of sugars and represents the greatest source of renewable hydrocarbon on earth. Unlike other renewable energy sources, biomass can be converted directly into liquid fuels. The two most common types of biofuels are ethanol (ethyl alcohol) and biodiesel. Ethanol is an alcohol, which can be produced by fermenting any biomass high in carbohydrates (starches, sugars, or celluloses) once fermentable sugars have been obtained from the biomass material. Sugars generated from degradation of plant biomass could provide plentiful, economically competitive feedstocks for fermentation to produce chemicals, plastics, and fuels or any other product of interest.

Fuel ethanol could be made from crops which contain starch such as feed grains, food grains, and tubers, such as potatoes and sweet potatoes. Crops containing sugar, such as sugar beets, sugarcane, and sweet sorghum also could be used for the production of ethanol. Sugar, in the form of raw or refined sugar, or as sugar in molasses requires no pre-hydrolysis (unlike corn starch) prior to fermentation. Consequently, the process of producing ethanol from sugar is simpler than converting corn starch into ethanol.

The yield and concentration of desired carbohydrates in plants are key determinants of the technical and economic feasibility of downstream industrial processes. However, the metabolic networks of plants for biosynthesis of sugars show substantial internal buffering and redundancy, with the consequence that alteration to a key gene in metabolism of a sugar commonly results in no useful change to the harvestable yield of the sugar (Moore, Australian Journal of Plant Physiology

22: 661-679 (1995); Nguyen-Quoc and Foyer, *J of Experimental Botany* 52: 881-889 (2001); Fernie et al., *Trends in Plant Science* 7: 35-41 (2002)).

SUMMARY OF THE INVENTION

Provided herein are methods for producing locked carbohydrates in a plant tissue by providing one or more carbohydrate-metabolizing enzymes that catalyze the conversion of an endogenous carbohydrate to a non-native carbohydrate. The invention encompasses plants and plant parts that produce one or more carbohydrate-metabolizing enzymes to yield a locked carbohydrate, with the consequence of increasing the total locked carbohydrate content in the plant. Further provided are hydrolytic enzymes (key enzymes) for converting the locked carbohydrate into a fermentable sugar. Fermentable sugars are used for a variety of industrial purposes including the production of ethanol.

DETAILED DESCRIPTION OF THE INVENTION

Overview

Plants accumulating large amounts of sugar are valuable as fermentation feedstocks for the downstream production of commercially-useful products. However, plants have various mechanisms to regulate the flow of sugars, therefore, sugar accumulation is limited in many plants. Plants contain both internal receptors and membrane-bound external receptors for monitoring sugar biosynthesis, transport, and uptake (reviewed in Lalonde et al. (1999) *Plant Cell* 11:707-726). Intracellular receptors modulate metabolic processes such as photosynthesis. Extracellular receptors sense external sugar concentrations in order to control sugar influx from the surrounding environment. Thus, the plant cells are capable of maintaining sufficient levels of sucrose by regulating metabolic processes and sugar uptake.

Provided herein is a method for producing locked storage carbohydrates in plants so that they cannot be metabolized by the plant. The methods comprise introducing into the plant or plant part one or more enzymes capable of converting an endogenous sugar into a locked carbohydrate. By "endogenous sugar" or "native sugar" is intended a sugar that is normally produced by a particular variety of plant. In contrast, a "locked carbohydrate" or a "locked sugar" is one that is not produced under normal conditions of growth or development of that variety of plant or in a particular plant part or plant organelle. Expression of an enzyme capable of converting the endogenous sugar into a locked carbohydrate (which is herein referred to as a "lock enzyme") in a plant will allow accumulation of the locked carbohydrates in the plant. Because these locked carbohydrates are not metabolized in plants, they are unlikely to be subject to "futile cycles" of degradation and synthesis in the mature storage tissues, which have the potential to decrease storage efficiency and harvestable yield. Many of these oligosaccharides, polysaccharides, or monosaccharides will also evade the plant's carbohydrate detecting mechanisms, such as sucrose sensing, such that native and non-native carbohydrate synthesis may occur to compensate for decreases in endogenous carbohydrates which have been diverted into the locked carbohydrate storage pathway.

Recently, Wu and Birch, *infra*, have demonstrated that converting sucrose to the non-metabolized sucrose isomer isomaltulose allows accumulation of isomaltulose and sucrose providing combined sugar production in sugarcane. Isomaltulose is currently used to manufacture sugar alcohols

consumed as low-calorie sweeteners (Schiweck et al. (1991) In F. W. Lichtenthaler (ed.), Carbohydrates as organic raw materials. Wiley-VCH, Weinheim, Germany), and it is an attractive renewable starting material for the manufacture of biosurfactants and biocompatible polymers (Lichtenthaler (2002) Accounts Chem. Res. 35:728-737).

The invention also comprises expressing hydrolytic enzymes capable of hydrolyzing the locked carbohydrates into fermentable sugars. These enzymes are herein referred to as "key enzymes." These enzymes may be of plant, bacterial, fungal, archeal, or other origin; may be provided exogenously in an enzyme preparation, may be expressed in a separate line of plants or the same line of plants, or in yeast or other microbes, or may be provided in microbes that are used in a fermentative process converting fermentable sugars, carbohydrates or di, tri, oligo or polymeric saccharides to useful fermentation products. Fermentable sugars are carbohydrates which can be metabolized by conventional organisms such as yeast. Fermentation is the process of energy production in a cell and is not limited to the production of alcohols. Fermentation refers to the breakdown and re-assembly of biochemicals for industry in either aerobic or anaerobic growth conditions. It generally is the process of energy production in a cell and is not limited to the production of alcohols. Commonly known fermentable sugars include but are not limited to sucrose, glucose and fructose.

Commercial applications of the invention include the production of sugarcane, sugar beet, or other plants capable of producing locked carbohydrates. In some embodiments, accumulation of the normal storage carbohydrates (e.g., sucrose) is not affected in these plants. These plants or their extracts are then treated with enzyme preparations or with microbes or plant materials expressing key enzymes capable of hydrolyzing locked carbohydrates into fermentable sugar. These sugars could then be used in fermentation for many purposes including ethanol production or any other product of interest.

Thus, the methods of the invention find particular use in the integration of current practices for the cultivation of crop plants for the purpose of obtaining a commercially desired plant material with increased accumulation of carbohydrates (locked or native) in a plant, and the use of the crop plant or plant part as a source of biomass for the production of fermentable sugars, or for agricultural and/or human consumption.

By a "crop plant" is intended any plant that is cultivated for the purpose of producing plant material that is sought after by man for either oral consumption, or for utilization in an industrial, pharmaceutical, or commercial process. The invention may be applied to any of a variety of plants, including, but not limited to maize, wheat, rice, barley, soybean, cotton, sorghum, oats, tobacco, strawberry, *Miscanthus* grass, Switch grass, trees, beans in general, rape/canola, alfalfa, flax, sunflower, safflower, millet, rye, sugarcane, sugar beet, cocoa, tea, *Brassica*, cotton, coffee, sweet potato, flax, peanut, clover; vegetables such as lettuce, tomato, cucurbits, cassava, potato, carrot, radish, pea, lentils, cabbage, cauliflower, broccoli, Brussels sprouts, peppers, and pineapple; tree fruits such as citrus, apples, pears, peaches, apricots, walnuts, avocado, banana, and coconut; and flowers such as orchids, carnations and roses.

As used herein, the term "plant part" or "plant tissue" includes plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of

plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, and the like.

The article "a" and "an" are used herein to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one or more element. Throughout the specification the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

"Isolated" means altered "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living animal in its natural state is not "isolated", but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs. A sequence is also isolated if separated from the chromosome and cell in which it naturally occurs in but inserted into a genetic context, chromosome, or cell in which it does not naturally occur.

Locked Carbohydrates

Sucrose is the major intermediary in carbon flux between source (photosynthetic) tissues and sink (growth and storage) tissues within plants, and it is the primary storage product in certain plants such as sugarcane and sugar beet. Plants have highly adapted sensors and transporters for sucrose, but it is generally considered that these sucrose sensors and transporters are not able to respond in the same way to locked carbohydrates (Loreti et al., Plant Physiol 123: 939-948 (2000); Sinha et al., Plant Physiol 128: 1480-1489 (2002)). In stark contrast with sucrose, plants are unable to metabolize these locked carbohydrates as a source of carbon and energy (Sinha et al., 2002).

While not bound by any particular theory or mechanism, specific alterations to metabolism, involving the conversion of a carbohydrate normally sensed by the plant into a locked carbohydrate that is not perceived in an equivalent manner, can shift metabolism and result in the accumulation of higher concentrations of locked carbohydrates or, in some cases, accumulation of higher concentrations of total carbohydrates.

Thus, provided herein are methods for the expression in a plant of an enzyme capable of converting an endogenous sugar into a locked sugar. The endogenous sugars produced by different plants may differ and as such an endogenous sugar of one plant may be non-native to another. Where the sugar is non-native to a particular plant, that plant is a candidate for production of a locked carbohydrate using the methods of the invention. Also, a non-native carbohydrate may also refer to a carbohydrate that is not normally produced in a particular subcellular compartment, or in a particular plant part of the native plant. In this embodiment, the subcellular compartment or the plant part would normally not be capable of metabolizing or transporting out of the compartment or plant part any non-native carbohydrate produced therein. Thus, it is essential to determine which carbohydrates are endogenously produced by a chosen plant or plant part to thereby deduce which carbohydrates are non-native to the plant and the type of carbohydrate-metabolizing enzyme(s) that could be useful for producing a locked carbohydrate in the plant.

For example, amylose (i.e., a type of starch) is a polysaccharide consisting of glucosyl residues linked by alpha-(1-4) bonds and is the primary carbohydrate storage compound found in most plants. Producing starch in plants that use sucrose as their primary carbohydrate storage compound, such as sugarcane, may permit the accumulation of starch which would behave as a "locked" sugar (i.e., sugar that cannot be metabolized by the plant).

The types of carbohydrates endogenously produced by plants can be determined using methods well known to persons of skill in the art. These methods include separation of sugars or sugar derivatives by electrophoresis or chromatography (including paper chromatography, thin layer chromatography, gas chromatography, gas-liquid chromatography and high-performance liquid chromatography) techniques. The separated components are typically identified by comparison of separation profiles with standards of known identity, or by analytical techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy. See, for example, reference may be made to Robinson 1980, *The Organic Constituents of Higher Plants*, Cordus Press, North Amherst, USA; Adams et al. 1999, *Anal. Biochem.* 266:77-84; Veronese and Perlot 1999, *Enz. Microbial Tech.* 24:263-269; Hendrix and Salvucci 2001, *J. Insect Physiol.* 47:423-432; Thompson et al. 2001, *Carbohydrate Res.* 331:149-161; each of which is incorporated by reference herein for their teachings regarding analysis of sugar content.

The endogenous or the non-native carbohydrates may include monosaccharides, oligosaccharides, sugar alcohols, sugar acids, amino sugars or other variants such as deoxy sugars, methyl sugars and the like. Examples of monosaccharides include compounds with formula $(CH_2O)_n$ where $n=3$ or more but suitably less than 10; including compounds comprising tetroses (e.g., erythrose, threose, erythrulose), pentoses (e.g., ribose, arabinose, xylose, lyxose, ribulose, xylulose), hexoses (e.g., allose, altrose, glucose, mannose, gulose, idose, galactose, talose, psicose, fructose, sorbose, tagatose), and longer molecules such as sedoheptulose or mannoheptulose. Oligosaccharides, which are formed by linking together two or more monosaccharide units through glycosidic bonds, may be selected from disaccharides (e.g., maltose, lactose, gentibiose, melibiose, trehalose, sophorose, primeverose, rutinose, sucrose, isomaltulose, trehalulose, turanose, maltulose, leucrose, 2-keto-sucrose) and longer oligomers such as raffinose, melezitose, isobemisiase or stachyose. Examples of sugar alcohols include, but are not limited to, erythritol, ribitol, mannitol, sorbitol. Non-limiting examples of sugar acids include gluconic acid, glucaric acid, glucuronic acid. Non-limiting examples of amino sugars include glucosamine, galactosamine. Endogenous or non-native sugars may also be selected from other variants such as deoxy sugars and methyl sugars. Further encompassed are isobemisiase, tagatose, isomaltotriose, dextrin, cyclodextrins, lactose, verbascose, amylose, and rhamnose.

Isomaltulose and Trehalulose

In certain embodiments, the locked carbohydrate is an isomer of the endogenous carbohydrate. In one example of this embodiment, the endogenous sugar is sucrose and the sugar-metabolizing enzyme is a sucrose isomerase, which converts the sucrose by isomerization to a locked sugar selected from isomaltulose and trehalulose. Isomaltulose .alpha.-D-glucopyranosyl-1,6-D-fructofuranose (also called palatinose) is a nutritive disaccharide, with sweetness and bulk similar to sucrose. Several characteristics make isomaltulose advantageous over sucrose for some applications in the food industry: 1) noncariogenic (not causing dental decay); 2) low glycemic index (useful for diabetics); 3) selec-

tive promotion of growth of beneficial bifidobacteria among human intestinal microflora; 4) greater stability of isomaltulose-containing foods and beverages; 5) less hygroscopic; 6) simple conversion into sugar alcohols with other useful properties as foods.

Sucrose isomerases (E.C. 5.4.99.11) are enzymes produced by organisms including various microbes, with the capability to convert the disaccharide sucrose into isomers such as isomaltulose (palatinose) or trehalulose. Sucrose isomerases vary in their properties including the disaccharide reaction products, the proportion of monosaccharides such as glucose and fructose in the reaction products, the kinetic properties of the enzymes, the optimal reaction conditions, and the sensitivity of the enzyme to variations from the optimal conditions (Veronese and Perlot, *Enzyme. Microb. Technol.* 24: 263-269 (1999)). An isolate of *Pantoea dispersa* designated UQ68J is exceptionally efficient in sucrose isomerase activity (Wu and Birch (2004) *J. Appl. Microbiol.* 97:93-103). Another exemplary sucrose isomerase has been isolated from *Erwinia carotovora* (GENBANK Accession No. YP049947).

Dextrans and Fructans

This invention also comprises transforming plants with one or more genes involved in the synthesis of fructans or dextrans. These genes may come from plant, bacterial, or fungal sources and should catalyze the formation of fructose and glucose polysaccharides or polysaccharides comprised of mixed sugars that are found in cane or sugar beet, sweet sorghum, mangel-wurzel or other sugar crops. The oligo- or polysaccharides produced may also comprise mixed sugar monomers, for example glucose, fructose, mannose and galactose.

By producing these fructan, dextran and mixed fructan and dextran carbohydrates in plants whose primary storage carbohydrate is sucrose, such as sugarcane and sugarbeet, a method for sequestering carbohydrates is provided in a form that is non-metabolizable for the plant. Such compounds may evade the sucrose sensing mechanisms of the plant so that they can be accumulated for later enzymatic hydrolysis to fermentable sugars.

Dextran is a collective name for high-molecular-weight polymers composed of D-glucose units connected with alpha-1,6 linkages and various amounts of side branches linked with alpha-1,2, alpha-1,3, or alpha-1,4 to the main chains. The enzymes that synthesize these glucans from sucrose are known by the generic term dextranucrase (1,6-alpha-D-glucan-6-alpha-glucosyltransferase, EC2.4.1.5.). The biosynthesis of dextran has been demonstrated in numerous bacteria, especially in *Streptococcus mutans*, *Leuconostoc mesenteroides* ssp. *mesenteroides* and *Leuconostoc mesenteroides* ssp. *dextranicum*. *Leuconostoc* produce the enzyme dextran sucrose and secrete it into the culture medium in the presence of sucrose. This enzyme, dextran sucrose, then synthesizes dextran from the sucrose substrate. Dextran has applications in several fields. It is used especially in biochemistry as a support for filtration chromatography on a gel of the Sephadex type. Additionally, in the field of therapeutics, it is used as a substitute for blood plasma (*Biochimie generale* (General Biochemistry)—J. H. WEIL—Masson, 6th edition—1990—p. 171).

Exemplary dextranucrase enzymes include (but are not limited to): the dextranucrase from *Streptococcus downei*, gtfS gene (Gilmore et al. (1990) *Infect. Immun.* 58 (8), 2452-2458; GENBANK Accession No. P29336); the dextranucrase from *Streptococcus mutans*, gtfI gene, produces a 1,3 glucose soluble dextran (Shiroza et al. (1987) *J. Bacteriol.* 169 (9), 4263-4270; GENBANK Accession No. P08987);

and the dextranase from *Streptococcus mutans* gtfD gene, gtfS protein (Terao et al. (1998) FEMS Microbiol. Lett. 161 (2), 331-336; GENBANK Accession No. P49331)

There is no common class of enzymes identified as "Leucrose synthases." Instead leucrose [O- α -D-glucopyranosyl-(1 \rightarrow 5)-D-fructopyranoside] is generally a byproduct of dextranase enzyme (EC 2.4.1.5) activity. These enzymes act as glucosyltransferases, and normally transfer a glucose unit hydrolyzed from a sucrose molecule to a growing dextran chain, or in the case of leucrose to a pyranosyl-fructose molecule yielding leucrose. Glucose can also serve as an acceptor for the transglycosylase reaction resulting in isomaltose (O- α -D-glucopyranosyl- α [1-6]- α -D-glucopyranoside) production. Since the 1950's leucrose has been made enzymatically typically using the *Leuconostoc mesenteroides* dextranase (The Preparation, Properties and Structure of the Disaccharide Leucrose Journal of the American Chemical Society, Stodola et. al; (1956) 78: 2415) followed by chemical purification.

Dextranases can be mutated to produce more leucrose and/or turanose. This has been shown for the dextranase of *Streptococcus oralis* (Engineering the Glucanase GTFR Enzyme Reaction and Glycosidic Bond Specificity: Toward Tailor-Made Polymer and Oligosaccharide Products, Biochemistry 2008, 47, 6678-6684, Hendrik Hellmuth et. al). Since dextranases can be mutated to produce leucrose it is reasonable to assume that other related enzymes (e.g. amylosucrase EC 2.4.1.4) or unrelated enzymes that also produce sucrose isomers could be mutated to produce leucrose. Leucrose synthase activity is attributed to any enzyme that produces leucrose by any mechanism, i.e. isomerization, transglycosylation, hydrolysis, dehydrogenation, reduction, etc.

The production of leucrose can be assayed using HPAE chromatography with pulsed amperometric detection (PAD). This technique is widely accepted as a preferred method for separating carbohydrates and is effective in separating sucrose isomers. Comparison of peak elution times with known standards is one method for determining the presence of leucrose. Full verification of the bond arrangements in the carbohydrate molecules can be determined either by methylation and acetylation of leucrose followed by GC MS, or directly by NMR spectroscopy if the samples are of sufficient quantity and purity.

Sucrose:sucrose fructosyltransferase (SST) (EC 2.4.1.99), 1,2- β -fructan 1-fructosyltransferase (FFT) (EC 2.4.1.100), 2- β -fructan 1-fructosyltransferase (FFT) (EC 2.4.1.100), glucan sucrose, and levan sucrose (EC 2.4.1.10) are enzymes within the larger class of fructosyl transferases. The fructosyl transferase enzymes catalyze the formation of fructans composed of fructose linked by β (2 \rightarrow 1) and/or β (2 \rightarrow 6) glucoside bonds. Fructosyl transferases may be identified and isolated from plant, bacterial, or fungal sources. These enzymes may be expressed in plants to accumulate fructans as storage carbohydrates. Accumulation of this polysaccharide (fructan) in sugarcane or other plants may allow the accumulation of excess carbohydrates.

Inulin is a fructan type carbohydrate polymer which occurs as a polydisperse composition in many plants and can also be produced by certain bacteria and fungi. Inulin from plant origin consists of a polydisperse composition of mainly linear chains composed of fructose units, mostly terminating in one glucose unit, which are linked to each other through β (2-1) fructosyl-fructose linkages.

Inulin molecules are synthesised by the concerted action of two enzymes: sucrose:sucrose 1-fructosyltransferase (in short 1-SST enzyme or 1-SST, used interchangeably) and

fructan:fructan 1-fructosyltransferase (in short 1-FFT enzyme or 1-FFT, used interchangeably) (Koops and Jonker, J of Experimental Botany 45: 1623-1631 (1994); and Koopos and Jonker, Plant Physiol 110: 1167-1175 (1996)). Both 1-SST and 1-FFT are active during the period of inulin synthesis and accumulation: 1-SST catalyzes the initial reaction of inulin biosynthesis, the conversion of sucrose into the smallest inulin molecule, the trisaccharide kestose (GFF). 1-FFT catalyzes the redistribution of terminal fructosyl units (-F) between inulin molecules, which results in a stepwise increase in chain length.

Amylose

This invention further comprises transforming plants with one or more genes involved in the synthesis of novel carbohydrates such as amylosucrase (E.C. 2.4.1.4) to produce amylose in order to accumulate carbohydrates for later fermentation into ethanol. Examples of enzymes that may catalyze the desired conversions include isomerases, epimerases, mutases, kinases, aldolases, transferases, transketolases, phosphatases, synthases, carboxylases, dehydrogenases and hydrolases. An exemplary amylosucrase includes the enzyme produced by *Neisseria polysacharea* (GENBANK Accession number Q9ZEU2), which catalyzes the conversion of sucrose to a linear α -1,4-linked glucan.

Alternan

Alternan is a polysaccharide consisting of glucosyl residues linked by alternate α -(1-3)/ α -(1-6) bonds. This polymer is highly soluble and has very low viscosity. Accumulation of this polysaccharide in sugarcane or other plants may allow the accumulation of excess carbohydrates.

Alternansucrase is an enzyme which catalyzes the conversion of sucrose to alternan. Alternansucrase is encoded by the *Asr* gene of *Leuconostoc mesenteroides* described in Jeannes et al. (1954) Am Chem Soc 76:5041-5052.

Key Enzymes

The invention also comprises expressing hydrolytic enzymes capable of hydrolyzing the locked carbohydrates into fermentable sugars. These enzymes are herein referred to as "key enzymes." These enzymes may be of plant, bacterial, fungal, archeal, or other origin; may be provided exogenously in an enzyme preparation, may be expressed in a separate line of plants or the same line of plants, or in yeast or other microbes, or may be provided in microbes that are used in a fermentative process to convert the locked carbohydrates into fermentable sugars. Yeast or microbes used in the fermentative process may also be identified or engineered to convert locked carbohydrates to energy. Furthermore, the locked carbohydrates may be converted to a fermentable sugar by chemical methods, e.g., by one or more chemicals capable of converting a locked carbohydrate into a fermentable sugar. The chemical(s) can be added prior to fermentation, or during the fermentation process.

Key enzymes can be isolated from, produced by, provided by a wide range of sources. Recombinant organisms such as plants, microbes or yeast, can be engineered to express a key enzyme. The recombinant organism can be used directly in a method of converting locked carbohydrates to fermentable sugars without further purification of the enzyme. Alternatively, key enzymes may be isolated from recombinant organisms for further use in the processing of locked carbohydrates. Native sources for key enzymes may also be used either directly (such as yeast or microbes which express a key enzyme normally) or by further isolation of the key enzyme. A key enzyme may be provided by a source selected from the group consisting of transgenic plant expressing one or more key enzymes, recombinant microbe expressing one or more key enzymes, transgenic yeast expressing one or more key

enzymes, microbe expressing one or more key enzymes, and yeast expressing one or more key enzymes.

Isomaltulose and trehalulose can be hydrolyzed by alpha-1,6-glucosidase enzymes. Exemplary glucosidase enzymes are set forth in SEQ ID NO:1-6 herein. Additional sequences are described in U.S. Pat. No. 5,786,140, and in Börnke et al. (2001) Journal of Bacteriology 183(8):2425-2430, each of which is herein incorporated by reference in its entirety.

Dextran-degrading enzymes form a diverse group of different carbohydrases and transferases. These enzymes have often been classified as endo- and exodextranases based on the mode of action and commonly called dextranases and include enzymes such as dextranases (EC3.2.1.11), glucan-1,6-alpha-D-glucosidases (EC3.2.1.70), glucan-1,6-alpha-isomaltosidases (EC3.2.1.94), dextran 1,6-alpha-isomaltotri-
 15 osidases (EC3.2.1.95), and branched-dextran exo-1,2-alpha-glucosidases (EC3.2.1.115)

Exodextranases, such as glucodextranase (EC3.2.1.70; glucan 1,6-alpha-glucosidase), catalyze stepwise hydrolysis of the reducing terminus of dextran and derived oligosaccharides to yield solely alpha-D-glucose; i.e., hydrolysis is accompanied by inversion at carbon-1 in such a way that new reducing ends are released only in the alpha-configuration. Some bacteria and yeasts are known to produce glucodextranases. Dextran-inducible extracellular glucodextranase occurs in *Arthrobacter globiformis* strains I42 and T-3044 (Oguma and Kobayashi (1996) J. Appl. Glycosci. 43:73-78; Oguma et al. (1999) Biosci. Biotechnol. Biochem. 63:2174-2182).

Intracellular dextran glucosidases (EC3.2.1.) producing alpha-D-glucose from dextran exist in several strains of *Streptococcus mitis* (Linder and Sund (1981) Caries Res. 15:436-444; Walker and Pulkownik (1973) Carbohydr. Res. 29:1-14; Walker and Pulkownik (1974) Carbohydr. Res. 36:53-66).

The soil bacterium *A. globiformis* T6 isomaltodextranase (EC3.2.1.94; 1,6-alpha-D-glucan isomaltohydrolase) is an extracellular exoenzyme capable of hydrolyzing dextran by removing successive isomaltose units from the nonreducing ends of the dextran chains (Sawai and Yano (1974) J. Biochem. 75:105-112; Sawai and Nawa (1976) Agric. Biol. Chem. 40:1246-1250).

Branched dextran exo-1,2-alpha-glucosidase (EC3.2.1.115) was found in the culture supernatant of the soil bacterium *Flavobacterium* sp. strain M-73 by Mitsubishi et al. (1979) Agric. Biol. Chem. 43:2283-2290. The enzyme had a strict specificity for 1,2-alpha-D-glucosidic linkage at the branch points of dextrans (containing 12 to 34% of 1,2-alpha linkages) and related polysaccharides producing free D-glucose as the only reducing sugar.

A list of additional exemplary microbial dextran-hydrolyzing enzymes and their substrate specificities and hydrolysis products is provided in Khalikova et al. (2005) Microbiology and Molecular Biology Reviews 2005:306-325, which is herein incorporated by reference as it describes and lists various dextran-hydrolyzing enzymes.

Fructanases are fructosydases which catalyze the hydrolysis of fructosidic linkages in fructans to break the fructan down into simpler sugar molecules. Fructans can be hydrolyzed to fermentable sugars through the catalytic activity of fructanases. For example, the fructanase 2,1-beta-D-fructan fructanohydrolase [EC 3.2.1.7] can hydrolyze fructan polymers into fructose monosaccharides which can be fermented to form ethanol.

Inulin can be converted to a fermentable carbohydrate using one or more inulase enzymes. Microbial inulinases (2,1-beta-D-fructan fructanohydrolase [EC 3.2.1.7]) are usually

inducible and exo-acting enzymes, which catalyze the hydrolysis of inulin by splitting off terminal fructosyl units (D-fructose).

Alternans can be hydrolyzed to form fermentable sugars by the activity of a alpha-1,6-glucosidase or alpha-1,3-glucosidase.

Methods

Provided herein are methods for improving the yield of carbohydrate in plants by expressing an enzyme capable of converting endogenous carbohydrate into locked carbohydrate. The locked carbohydrates accumulated in the plants described herein can be converted to fermentable carbohydrates using one or more of the key enzymes disclosed herein, which can then be used as fermentation feedstocks for ethanol, propanol, butanol or other fuel alcohol, ethanol-containing beverages (such as malted beverages and distilled spirits), and other fermentation products such as foods, nutraceuticals, enzymes and industrial materials. The methods for fermentation using plant-derived carbohydrate feedstocks are well known to those skilled in the art, with established processes for various fermentation products (see for example Vogel et al. 1996, Fermentation and Biochemical Engineering Handbook: Principles, Process Design, and Equipment, Noyes Publications, Park Ridge, N.J., USA and references cited therein). Key enzyme proteins could also be incorporated into the ethanol production process downstream of the feedstock step. It is envisioned that locked carbohydrates could be harvested and, in the process of making ethanol, the key enzyme is added during the production process. Key enzyme proteins could also be incorporated into the fermentable sugar production process downstream of the feedstock step. It is envisioned that locked carbohydrates could be harvested and, in the process of making fermentable sugar, the key enzyme is added during the production process.

In one embodiment, the use of the methods disclosed herein results in a substrate that leads to higher ethanol yields compared to the ethanol yield from plant material not accumulating locked carbohydrates. The increase in ethanol yield can be at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 20%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, or greater. Even small increases in ethanol yield will translate to large volumes of ethanol produced over time in a commercial-scale fermentation process. Such improvements in ethanol production could result in a significant increase in profit to the ethanol producer.

In one embodiment, the use of the methods disclosed herein results in a substrate that leads to higher carbohydrate yields compared to the carbohydrate yield from plant material not accumulating locked carbohydrates. The increase in carbohydrate yield can be at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 20%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, or greater. Even small increases in carbohydrate yield will translate to large volumes of carbohydrate produced over

time in a commercial-scale fermentation process. The carbohydrate may be sucrose or a combination of sucrose and a locked sugar.

In another embodiment, the plants accumulating locked carbohydrates can be used in various other downstream products other than ethanol production. Locked carbohydrates can be converted into fermentable sugars which are used in many commercial fermentation processes including growing recombinant yeast which produce important chemicals such as insulin, antibodies, or enzymes. Isomaltulose is currently used to manufacture sugar alcohols consumed as low-calorie, non-cariogenic sweeteners. Fructose also has value as a sweetener in high fructose syrups such as high fructose corn syrup. Plants engineered to produce fructans as a locked sugar may be used as a source of fructans which, after hydrolysis by a fructanase enzyme, produce a solution with a high fructose concentration. In such plants the yield of fructan may be increased by expressing an additional enzyme (e.g., glucose isomerase) to catalyze the conversion of glucose to fructose. The glucose isomerase (invertase) could be expressed in maize endosperm, or expressed in microbes. The purified enzyme could be used to produce fructans, glucans and alternans.

Sweeter plant products can be generated by expressing in plants a combination of enzymes that first allow for the accumulation of fructans in the plant and then convert the fructans directly or indirectly to fructose. Expressing invertase (glucose isomerase) in plants accumulating fructans will lead to a higher sweetness index in the plant.

In another embodiment, plants accumulating locked carbohydrates as described herein are useful for providing protection of the plant against disease. While not being bound by any particular theory or mechanism, plants accumulating locked sugars may be more tolerant or resistant to microbial infection due to the presence of carbohydrates other than sucrose, since infection by some microbes depends upon the content of sucrose in the plant.

Enzyme Extracts for Key Enzyme

In various embodiments of the present invention, the enzyme capable of converting the locked carbohydrate to a fermentable carbohydrate (referred to herein as the "key" enzyme) is provided as a purified or partially-purified preparation of the enzyme. The exogenously-added key enzyme may be de novo synthesized, or may be isolated from an organism expressing the enzyme prior to addition of the enzyme to the locked carbohydrate-containing plant material.

A purified or semi-purified preparation of enzyme will contain at least one class of key enzyme, but may also contain one or more additional enzymes of the same or different class. The preparation may further comprise one or more additional enzymes useful in the starch conversion method, such as amylase or glucoamylase. A "semi-purified" enzyme preparation will contain one or more key enzymes, one or more additional enzymes useful in the starch conversion process, or may contain other buffers or stabilizing agents (e.g., glycerol). Furthermore, the semi-purified enzyme preparation may also be culture supernatant or crude extract collected from a cell population expressing and/or secreting the enzyme. The preparation may also be a lyophilized formulation of enzyme that is reconstituted upon addition to the locked carbohydrate-containing plant material.

The various key enzymes discussed herein can be expressed in and isolated from any number of eukaryotic and prokaryotic organisms. Appropriate expression cassettes, vectors, transformation, and transfection techniques for a particular organism of interest will be evident to one of skill in the art.

In one embodiment, bacterial cells, such as *E. coli*, *Streptomyces*, *Bacillus subtilis*; and various species within the genera *Escherichia*, *Pseudomonas*, *Serratia*, *Streptomyces*, *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Microbacterium*, and *Staphylococcus* can be used as a host to express one or more classes of key enzymes encompassed herein. Methods for transformation of bacterial hosts are described in, for example, U.S. Patent Publication No. 2003/0135885.

In another embodiment, fungal hosts, such as fungal host cells belonging to the genera *Aspergillus*, *Rhizopus*, *Trichoderma*, *Neurospora*, *Mucor*, *Penicillium*, etc., such as yeast belonging to the genera *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Trichosporon*, *Schwanniomyces*, etc. may be used. Transformation of fungus may be accomplished according to Gonni et al. Agric. Biol. Chem., 51:2549 (1987).

Another suitable host includes any number of eukaryotic cells, for example, insect cells such as *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS or Bowes melanoma, C127, 3T3, CHO, HeLa and BHK cell lines. Any host can be used insofar as it can express the gene of interest. The American Type Culture Collection maintains cell lines from a wide variety of sources and many of these cultures can be used to generate a transgenic cell line capable of expressing a heterologous enzyme. Transformation vectors appropriate for eukaryotic cells are available commercially such as pXT1, pSG5 (Stratagene) pSVK3, pBPV, pMSG, and pSV-LSV40 (Pharmacia). Techniques for transformation and selection of transgenic eukaryotic cells are well known in the art. Exemplary methods are also described elsewhere herein.

In another embodiment, the key enzymes can be isolated from an organism that endogenously expresses the enzyme, or the organism expressing the enzyme can be used in one or more fermentation steps without the need for purification or isolation of the enzyme from the organism.

Additional methods for generating an enzyme extract are described in, for example, Conrad et al. (1995) *Eur. J. Biochem.* 230, 481-490; Chiang et al. (1979) *Starch* 31 Nr.3, S.86-92; Schwardt, E. (1990) *Food Biotechnology*, 4(1), 337-351; Morgan and Priest (1981) *Journal of Applied Bacteriology* 50, 107-114; Laderman et al. (1993) *Journal of Biological Chemistry* Vol. 268, No. 32, pp. 24394-24401, each of which is herein incorporated by reference in its entirety.

Transgenic Plants

In one embodiment of the present invention, the locked carbohydrate-containing plant material comprises plant parts derived from at least one variety of a transgenic plant expressing at least one polynucleotide encoding a lock enzyme. In another embodiment, the transgenic plant material expresses more than one lock enzyme, resulting in the accumulation of more than one type of locked carbohydrate. In yet another embodiment, both the lock and the key enzymes are expressed in plant material. Where both the lock and the key enzymes are provided as transgenic plant material, each class of enzyme may be expressed in the same plant variety, or may be expressed in different plant varieties.

As used herein the term "transgenic" refers to plants that include an exogenous polynucleotide (e.g., gene) that is stably maintained in the transformed plant and is stably inherited by progeny in successive generations. The term "transgenic plant" can refer either to the initially transformed plant or to the progeny of the initially transformed plant. Techniques for transforming plants, plant cells or plant tissues can include, but are not limited to, transformation with DNA employing *A. tumefaciens* or *A. rhizogenes* as the transforming agent, electroporation, DNA injection, microprojectile bombardment, and particle acceleration. See, for example, EP 295959 and EP 138341. As used herein, the terms "plant material" or

“plant part” includes plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, tubers, rhizomes and the like.

Where both the lock and the key enzymes are provided by transgenic plant material, it is not necessary for the plant material expressing the key enzyme to be 100% transgenic for the key enzyme. Rather, it is only necessary for the plant material to contain an amount of key enzyme that is sufficient for the downstream use (e.g., for conversion of locked carbohydrates to fermentable sugars). For example, for fermentation purposes, a sufficient amount of the key enzyme may be provided in the fermentation process by less than 100% key enzyme-expressing plant material. For example, a sufficient amount of key enzyme may be provided to the fermentation process when only about 0.1% of the locked carbohydrate-containing plant material expresses the key enzyme, or only about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, or about 20%, of the plant material. However, it is contemplated that the percentage of plant material expressing the key enzyme could be as much as 100%, including, for example, about 25%, about 30%, about 35%, about 40%, about 50%, about 60%, about 65%, about 70%, about 80%, about 90%, about 95%, or about 99% of the plant material.

The methods of the invention are particularly useful in plants producing high amounts of sugar, such as (for example), sugarcane, sugar beet, and sorghum. However, the plant material can be derived from any plant, including but not limited to plants producing edible flowers such as cauliflower (*Brassica oleracea*), artichoke (*Cynara scolymus*), and safflower (*Carthamus*, e.g. *tinctorius*); fruits such as apple (*Malus*, e.g. *domesticus*), banana (*Musa*, e.g. *acuminata*), berries (such as the currant, *Ribes*, e.g. *rubrum*), cherries (such as the sweet cherry, *Prunus*, e.g. *avium*), cucumber (*Cucumis*, e.g. *sativus*), grape (*Vitis*, e.g. *vinifera*), lemon (Citrus limon), melon (*Cucumis melo*), nuts (such as the walnut, *Juglans*, e.g. *regia*; peanut, *Arachis hypogaeae*), orange (Citrus, e.g. *maxima*), peach (*Prunus*, e.g. *persica*), pear (*Pyrus*, e.g. *communis*), pepper (*Solanum*, e.g. *capsicum*), plum (*Prunus*, e.g. *domestica*), strawberry (*Fragaria*, e.g. *moschata*), tomato (*Lycopersicon*, e.g. *esculentum*); leafy, such as alfalfa (*Medicago*, e.g. *sativa*), sugar cane (*Saccharum*), cabbages (such as *Brassica oleracea*), endive (*Cichorium*, e.g. *endivia*), leek (*Allium*, e.g. *porrum*), lettuce (*Lactuca*, e.g. *sativa*), spinach (*Spinacia* e.g. *oleraceae*), tobacco (*Nicotiana*, e.g. *tabacum*); roots, such as arrowroot (*Maranta*, e.g. *arundinacea*), beet (*Beta*, e.g. *vulgaris*), carrot (*Daucus*, e.g. *carota*), cassava (*Manihot*, e.g. *esculenta*), turnip (*Brassica*, e.g. *rapa*), radish (*Raphanus*, e.g. *sativus*) yam (*Dioscorea*, e.g. *esculenta*), sweet potato (*Ipomoea batatas*); seeds, such as bean (*Phaseolus*, e.g. *vulgaris*), pea (*Pisum*, e.g. *sativum*), soybean (Glycine, e.g. *max*), wheat (*Triticum*, e.g. *aestivum*), barley (*Hordeum*, e.g. *vulgare*), corn (*Zea*, e.g. *mays*), rice (*Oryza*, e.g. *sativa*); grasses, such as *Miscanthus* grass (*Miscanthus*, e.g., *giganteus*) and switchgrass (*Panicum*, e.g. *virgatum*); trees such as poplar (*Populus*, e.g. *tremula*), pine (*Pinus*); shrubs, such as cotton (e.g., *Gossypium hirsutum*); and tubers, such as kohlrabi (*Brassica*, e.g. *oleraceae*), potato (*Solanum*, e.g. *tuberosum*), and the like.

The locked carbohydrate-containing plant material may also comprise one or more varieties of plants having naturally-occurring genetic variability resulting in altered starch

metabolism. Many such plants carry mutations in genes encoding isoforms of starch synthesis or starch degradation enzymes. For example, plants have been identified which are heterozygous or homozygous for one or more of the waxy (wx), amylose extender (ae), dull (du), horny (h), shrunken (sh), brittle (bt), floury (fl), opaque (O), or sugary (su) mutant alleles. See, for example, U.S. Pat. Nos. 4,428,972; 4,767,849; 4,774,328; 4,789,738; 4,789,557; 4,790,997; 4,792,458; 4,798,735; and 4,801,470, herein incorporated by reference.

Dual Expression of Lock Enzymes

The invention also comprises the simultaneous expression of two lock enzymes such as two sucrose isomerases, one that produces predominantly isomaltulose, and one that produces predominantly trehalulose, so that both isomers of sucrose may be accumulated in the same plant. Sugarcane possesses an excess capacity for carbohydrate synthesis, however, there is a continuous “futile cycle” of sucrose synthesis and breakdown in sugarcane. By diverting carbohydrates into a form that is not metabolized by the plant, these carbohydrates may be removed from that futile cycle, and the plant may make up for the loss by producing more sucrose. The fact that Wu and Birch have seen isomaltulose accumulate to the same level as sucrose, without decreasing the amount of sucrose, suggests that this excess capacity of sugarcane for sugar synthesis has not been exhausted. By genetically modifying sugarcane with two or more lock enzymes that produce more than one isomers of sucrose (isomaltulose, trehalulose, leucrose, etc.) at equivalent levels it may be possible to significantly increase the total sugar content in sugarcane, or to increase the level of locked sugar in the sugarcane.

In one embodiment, the total carbohydrate content, or the total locked carbohydrate content, or both, is increased at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 125%, at least about 150%, at least about 2-fold, at least about 3-fold, at least about 4-fold or greater when compared to the same variety of plant that does not accumulate locked carbohydrate according to the methods of the invention.

Sucrose isomerase enzymes producing predominantly isomaltulose include, for example, the *P. dispersa* UQ68J enzyme described in U.S. Pat. No. 7,250,282, which is herein incorporated by reference in its entirety. Other enzymes producing predominantly trehalulose include, for example, the whitefly enzyme characterized by Salvucci (2003) Comp. Biochem. Physiol. B 135:385-395. While not to be limited by theory, the whitefly enzyme may be a representative of the lock enzyme trehalulose synthase.

Subcellular Targeting

For the purpose of producing starch in a transgenic plant, it may be advantageous to target the lock enzyme in the plant to subcellular compartments that have high concentrations of sucrose, such as the vacuole of sugarcane. Another target may be the vacuole of the maize endosperm. Targeting an enzyme capable of synthesizing starch from sucrose to the vacuole of maize endosperm cells may permit the accumulation of more starch in the maize endosperm as naturally occurring enzymes do not produce starch in the vacuoles of maize endosperm cells. Alternatively targeting to the apoplast is another way to achieve conversion of sucrose into locked sugars such as starch or isomaltulose. In plants such as maize, sucrose accumulates in the leaf and is transported to the ear during grain filling which provides a carbon sink.

In one embodiment, the lock enzyme is targeted to the amyloplast, where locked carbohydrate can accumulate, and the key enzyme (when expressed in the same plant) is targeted to the apoplast. The key enzyme can be targeted to the apoplast using, for example, the maize Gamma zein N-terminal

signal sequence, which confers apoplast-specific targeting of proteins. The lock enzyme may be targeted to the amyloplast by, for example, fusion to the waxy amyloplast targeting peptide (Klosgen et al., 1986) or to a starch granule. For example, the polynucleotide encoding the lock enzyme may be operably linked to a chloroplast (amyloplast) transit peptide (CTP) and a starch binding domain, e.g., from the waxy gene.

Directing the key enzyme to the apoplast will allow the enzyme to be localized in a manner that it will not come into contact with the locked carbohydrate substrate. In this manner the enzymatic action of the enzyme will not occur until the enzyme contacts its substrate. The enzyme can be contacted with its substrate by the process of milling (physical disruption of the cell integrity), or heating the cells or plant tissues to disrupt the physical integrity of the plant cells or organs that contain the enzyme. For example the key enzyme can be targeted to the apoplast or to the endoplasmic reticulum so as not to come into contact with the locked carbohydrate in the amyloplast. Milling of the grain will disrupt the integrity of the grain and the key enzyme will then contact the starch granules. In this manner the potential negative effects of co-localization of an enzyme and the locked carbohydrate can be circumvented.

Locked Carbohydrates as Selectable Markers

Plant transformation requires the use of positive selectable marker genes for identification and propagation of transformed tissue and the elimination of non-transformed tissue. One advantage of this system would be the ability to select and/or screen for expression and/or accumulation of the key enzyme involved in the breakdown of the locked carbohydrates, from the very earliest stages of the plant transformation process. A transformation system using the desired enzyme end product as a means of initial selection would permit early screening for position effects or genomic insertion sites that lead to high level or constitutive expression of the transgene. Also, the use of the desired end product as the selectable marker can reduce the number of genes that must be transferred into the plant. This will reduce the size of the T-DNA needed for transformation and be useful in the production of "molecular stacks" in which multiple transgenes are desired in a single transgenic plant, i.e., eliminate the need for an extraneous selectable marker gene such as PMI, or antibiotic resistance genes that are necessary for production of transgenic plants, but are no longer useful to the plant after transformation/selection. However, it is contemplated that multiple selectable markers can be used in the methods of the invention, including those used solely for selection.

In one embodiment, an alpha-1,6-glucosidase enzyme may be used to cleave the alpha-1,6-glucoside linkage between glucose and fructose in the disaccharide isomaltulose. This enzyme is desirable for converting isomaltulose produced by transgenic sugarcane plants into fermentable sugar or ethanol and may be useful as a novel selectable marker for sugarcane transformation.

Expression Cassettes

A plant or plant part expressing a lock and/or key enzyme can be obtained by introducing into the plant or plant part a heterologous nucleic acid sequence encoding the enzyme. The heterologous nucleic acid sequences may be present in DNA constructs or expression cassettes. "Expression cassette" as used herein means a nucleic acid molecule capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operatively linked to the heterologous nucleotide sequence of interest (i.e., lock and/or key enzyme) which is operatively linked to termination signals. It also typically comprises sequences

required for proper translation of the nucleotide sequence. The expression cassette comprising the lock and/or key enzyme may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is heterologous with respect to the host. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter that initiates transcription only when the host cell is exposed to some particular external stimulus. Additionally, the promoter can also be specific to a particular tissue or organ or stage of development.

The expression cassette may optionally comprise a transcriptional and translational termination region (i.e. termination region) functional in plants. In some embodiments, the expression cassette comprises a selectable marker gene to allow for selection for stable transformants. Expression constructs of the invention may also comprise a leader sequence and/or a sequence allowing for inducible expression of the lock and/or key enzyme. See, Guo et al. (2003) *Plant J.* 34:383-92 and Chen et al. (2003) *Plant* 3.36:731-40 for examples of sequences allowing for inducible expression.

The regulatory sequences of the expression construct are operably linked to the nucleic acid sequence encoding the lock and/or key enzyme. By "operably linked" is intended a functional linkage between a first sequence and a second sequence for instance, the first sequence may be a promoter sequence which is operably linked to a second sequence wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleotide sequences being linked are contiguous; however, the sequences may have linking sequences that join them together, thus the operably linked sequences may not be directly linked.

Promoter

Any promoter capable of driving expression in the plant of interest may be used in the practice of the invention. The promoter may be native or analogous or foreign or heterologous to the plant host. The terms "heterologous" and "exogenous" when used herein to refer to a nucleic acid sequence (e.g. a DNA or RNA sequence) or a gene, refer to a sequence that originates from a source foreign to the particular host cell or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell but has been modified through, for example, the use of DNA shuffling. The terms also include non-naturally occurring multiple copies of a naturally occurring DNA sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is not ordinarily found. Exogenous DNA segments are expressed to yield exogenous polypeptides.

The choice of promoters to be included depends upon several factors, including, but not limited to, efficiency, selectability, inducibility, desired expression level, and cell- or tissue-preferential expression. For example, where expression in specific tissues or organs is desired, tissue-specific promoters may be used. In contrast, where gene expression in response to a stimulus is desired, inducible promoters are the regulatory elements of choice. Where continuous expression is desired throughout the cells of a plant, constitutive promoters are utilized. It is a routine matter for one of skill in the art

to modulate the expression of a sequence by appropriately selecting and positioning promoters and other regulatory regions relative to that sequence.

A number of plant promoters have been described with various expression characteristics. Examples of some constitutive promoters which have been described include the rice actin 1 (Wang et al., *Mol. Cell. Biol.*, 12:3399 (1992); U.S. Pat. No. 5,641,876), CaMV 35S (Odell et al., *Nature*, 313:810 (1985)), CaMV 19S (Lawton et al., 1987), nos (Ebert et al., 1987), Adh (Walker et al., 1987), sucrose synthase (Yang & Russell, 1990), and the ubiquitin promoters.

Vectors for use in tissue-specific targeting of genes in transgenic plants will typically include tissue-specific promoters and may also include other tissue-specific control elements such as enhancer sequences. Promoters which direct specific or enhanced expression in certain plant tissues will be known to those of skill in the art in light of the present disclosure. These include, for example, the *rbcS* promoter, specific for green tissue; the *ocs*, *nos* and *smas* promoters which have higher activity in roots or wounded leaf tissue; a truncated (−90 to +8) 35S promoter which directs enhanced expression in roots, an α -tubulin gene that directs expression in roots and promoters derived from zein storage protein genes which direct expression in endosperm.

Tissue specific expression may be functionally accomplished by introducing a constitutively expressed gene (all tissues) in combination with an antisense gene that is expressed only in those tissues where the gene product is not desired.

Moreover, several tissue-specific regulated genes and/or promoters have been reported in plants. Some reported tissue-specific genes include the genes encoding the seed storage proteins (such as napin, cruciferin, beta-conglycinin, and phaseolin) zein or oil body proteins (such as oleosin), or genes involved in fatty acid biosynthesis (including acyl carrier protein, stearoyl-ACP desaturase, and fatty acid desaturases (fad 2-1)), and other genes expressed during embryo development (such as *Bce4*, see, for example, EP 255378 and Kridl et al., *Seed Science Research*, 1:209 (1991)). Examples of tissue-specific promoters, which have been described include the lectin (Vodkin, *Prog. Clin. Biol. Res.*, 138: 87 (1983); Lindstrom et al., *Der. Genet.*, 11:160 (1990)), corn alcohol dehydrogenase 1 (Vogel et al., *EMBO J.*, 11:157 (1989); Dennis et al., *Nucleic Acids Res.*, 12:3983 (1984)), corn light harvesting complex (Simpson, 1986; Bansal et al., *Proc. Natl. Acad. Sci. USA*, 89:3654 (1992)), corn heat shock protein (Odell et al., *Nature*, 313: 810 (1985)); pea small subunit RuBP carboxylase ((Poulsen et al., *Mol. Gen. Genet.* 205:193 (1986)); *Ti* plasmid mannopine synthase ((Langridge et al., *Cell* 34:1015 (1989)), *Ti* plasmid nopaline synthase ((Langridge et al., *Cell* 34:1015 (1989)), petunia chalcone isomerase (vanTunen et al., *EMBO J.*, 7: 1257 (1988)), bean glycine rich protein 1 (Keller et al., *Genes Dev.*, 3:1639 (1989)), truncated CaMV 35S (Odell et al., *Nature*, 313:810 (1985)), potato patatin (Wenzler et al., *Plant Mol. Biol.*, 13:347 (1989)), root cell (Yamamoto et al., *Nucleic Acids Res.*, 18:7449 (1990)), maize zein (Reina et al., *Nucleic Acids Res.*, 18:6425 (1990); Kriz et al., *Mol. Gen. Genet.*, 207:90 (1987); Wandelt et al., *Nucleic Acids Res.*, 17:2354 (1989); Langridge et al., *Cell*, 34:1015 (1983); Reina et al., *Nucleic Acids Res.*, 18:7449 (1990)), globulin-1 (Belanger et al., *Genetics*, 129:863 (1991)), α -tubulin, *cab* (Sullivan et al., *Mol. Gen. Genet.*, 215:431 (1989)), PEPCase ((Hudspeth et al., *Plant Mo. Bio.*, 12:579 (1989)), *R* gene complex-associated promoters (Chandler et al., *Plant Cell*, 1:1175 (1989)), and chalcone synthase promoters (Franken et al., *EMBO J.*, 10:2605 (1991)). Particularly useful for seed-specific expres-

sion is the pea vicilin promoter (Czako et al., *Mol. Gen. Genet.*, 235:33 (1992). (See also U.S. Pat. No. 5,625,136, herein incorporated by reference.) Other useful promoters for expression in mature leaves are those that are switched on at the onset of senescence, such as the *SAG* promoter from *Arabidopsis* (Gan et al., *Science*, 270:1986 (1995)).

In various embodiments, the lock and/or key enzyme is active in the fruit of the plant. A class of fruit-specific promoters expressed at or during anthesis through fruit development, at least until the beginning of ripening, is discussed in U.S. Pat. No. 4,943,674, the disclosure of which is hereby incorporated by reference. cDNA clones that are preferentially expressed in cotton fiber have been isolated (John et al., *Proc. Natl. Acad. Sci. USA*, 89:5769 (1992)). cDNA clones from tomato displaying differential expression during fruit development have been isolated and characterized (Mansson et al., *Gen. Genet.*, 200:356 (1985); Slater et al., *Plant Mol. Biol.*, 5:137 (1985)). The promoter for polygalacturonase gene is active in fruit ripening. The polygalacturonase gene is described in U.S. Pat. No. 4,535,060, U.S. Pat. No. 4,769,061, U.S. Pat. No. 4,801,590, and U.S. Pat. No. 5,107,065, which disclosures are incorporated herein by reference. The fruit specific E8 promoter is described in Deikman et al. (1988, *EMBO J.* 2: 3315-3320) and DellaPenna et al. (1989, *Plant Cell* 1: 53-63). In another embodiment, promoters that selectively express coding sequences in sucrose storage tissues (such as the mature stems of sugarcane and the tubers of sugar beet) may be used. For example, promoters specific for the mature stems of sugarcane are described in International Publication WO 01/18211.

In another embodiment, the expression of the lock enzyme is under the control of a sink tissue-specific promoter. By "sink tissue-specific promoter" is meant a promoter that preferentially directs expression of an operably linked transcribable sequence in the sink tissue of a plant as compared to expression in other tissues of the plant, including source tissues (e.g., leaf). "Sink cell" and "sink tissue" as used herein, refer to cells, tissues or organs which at the time of harvest comprise organic carbon that has entered the cells by net inflow in a form other than carbon dioxide. In plants, sink tissues include all non-photosynthetic tissues, as well as photosynthetic tissues with a net inflow of organic carbon fixed by other photosynthetic cells or otherwise obtained from the surrounding medium or environment by means other than direct fixation of carbon dioxide.

Other examples of tissue-specific promoters include those that direct expression in leaf cells following damage to the leaf (for example, from chewing insects), in tubers (for example, patatin gene promoter), and in fiber cells (an example of a developmentally-regulated fiber cell protein is E6 (John et al., *Proc. Natl. Acad. Sci. USA*, 89:5769 (1992)). The E6 gene is most active in fiber, although low levels of transcripts are found in leaf, ovule and flower. Other tissue-specific promoters can be isolated by one skilled in the art (see U.S. Pat. No. 5,589,379).

Several inducible promoters have been reported. Many are described in a review by Gatz, in *Current Opinion in Biotechnology*, 7:168 (1996) and Gatz, C., *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48:89 (1997). Examples include tetracycline repressor system, Lac repressor system, copper-inducible systems, salicylate-inducible systems (such as the PR1a system), glucocorticoid-inducible (Aoyama T. et al., *N—H Plant Journal*, 11:605 (1997)) and ecdysone-inducible systems. Other inducible promoters include ABA- and turgor-inducible promoters, the promoter of the auxin-binding protein gene (Schwob et al., *Plant J.*, 4:423 (1993)), the UDP glucose flavonoid glycosyl-transferase gene promoter (Ralston et al.,

Genetics, 119:185 (1988)), the MPI proteinase inhibitor promoter (Cordero et al., Plant J., 6:141 (1994)), and the glyceraldehyde-3-phosphate dehydrogenase gene promoter (Kohler et al., Plant Mol. Biol., 29: 1293 (1995); Quigley et al., J. Mol. Evol., 29:412 (1989); Martinez et al., J. Mol. Biol., 208:551 (1989)). Also included are the benzene sulphonamide-inducible (U.S. Pat. No. 5,364,780) and alcohol-inducible (WO 97/06269 and WO 97/06268) systems and glutathione S-transferase promoters.

Other studies have focused on genes inducibly regulated in response to environmental stress or stimuli such as increased salinity, drought, pathogen and wounding. (Graham et al., J. Biol. Chem., 260:6555 (1985); Graham et al., J. Biol. Chem., 260:6561 (1985), Smith et al., Planta, 168:94 (1986)). Accumulation of metallocarboxypeptidase-inhibitor protein has been reported in leaves of wounded potato plants (Graham et al., Biochem. Biophys. Res. Comm., 101:1164 (1981)). Other plant genes have been reported to be induced by methyl jasmonate, elicitors, heat-shock, anaerobic stress, or herbicide safeners.

Preferably, in the case of a multicellular organism, the promoter can also be specific to a particular tissue, organ or stage of development. Examples of such promoters include, but are not limited to, the *Zea mays* ADP-gpp and the *Zea mays* Gamma zein promoter and the *Zea mays* globulin promoter.

Expression of a gene in a transgenic plant may be desired only in a certain time period during the development of the plant. Developmental timing is frequently correlated with tissue specific gene expression. Timing the expression of carbohydrate-metabolizing enzymes advantageously takes into consideration the change in carbohydrate concentration that occurs during plant development. The importance of a carbohydrate within tissue may also change with time and, in this regard, sink tissue may undergo changes in sucrose concentrations during development. For example, sucrose concentration in certain fruits such as sweet melons changes as the fruit matures. Hexose sugars accumulate early in development, followed by high levels of sucrose at later stages (Schaffer et al., 1987, Phytochemistry 26: 1883-1887). In developing corn endosperm, sucrose concentration increases from 8 to 12 days after pollination and then drops more than ten fold 28 days after pollination (Tsai et al., 1970, Plant Phys. 46: 299-306). Additionally, sucrose concentration in soybean seed changes significantly during development as raffinose saccharides content increases dramatically, 53 days after anthesis (Amuti, 1977, Phytochemistry 16: 529-532). In pea seed, sucrose content falls dramatically with continued development (Holl and Vose, Can. 1980, J. Plant Sci. 60: 1109-1114). These examples illustrate the desirability of promoter selection for specific expression of an enzyme gene timed to take advantage of fluctuating sucrose pools. Thus, in various embodiments, the promoter is an inducible promoter which is capable of driving expression of the enzyme-encoding polynucleotide at an appropriate developmental stage of the plant. In this embodiment, the transcriptional control element is suitably a developmentally regulated promoter to control the timing of expression.

Localization Signals

The polynucleotide sequences encoding the lock and/or key enzyme of the present invention may be operably linked to polynucleotide sequences encoding localization signals or signal sequence (at the N- or C-terminus of a polypeptide), e.g., to target the enzyme to a particular compartment within a plant. Examples of such targets include, but are not limited to, the vacuole, endoplasmic reticulum, chloroplast, amyloplast, starch granule, or cell wall, or to a particular tissue, e.g.,

seed. The expression of a polynucleotide encoding a lock and/or key enzyme having a signal sequence in a plant, in particular, in conjunction with the use of a tissue-specific or inducible promoter, can yield high levels of localized enzyme in the plant. Targeting or signal sequences can be used to localize a lock or key enzyme such that the enzyme does not come into contact with a specific substrate during the growth and development of the plant. For instance, key enzymes expressed in plants that accumulate locked sugars may be targeted away from the plant organelle or compartment which contains the locked sugar. At the time of harvest, the plant tissue may be physically disrupted in order to combine the key enzyme with the locked sugar during the processing of the plant tissue.

Thus, vectors may be constructed and employed in the intracellular targeting of a specific gene product within the cells of a transgenic plant or in directing a protein to the extracellular environment. This will generally be achieved by joining a DNA sequence encoding a transit or signal peptide sequence to the coding sequence of a particular gene. The resultant transit, or signal, peptide will transport the protein to a particular intracellular or extracellular destination, respectively, and will then be post-translationally removed. Transit or signal peptides act by facilitating the transport of proteins through intracellular membranes, e.g., vacuole, vesicle, plastid and mitochondrial membranes, whereas signal peptides direct proteins through the extracellular membrane.

Numerous signal sequences are known to influence the expression or targeting of a polynucleotide to a particular compartment or outside a particular compartment. Suitable signal sequences and targeting promoters are known in the art and include, but are not limited to, those provided herein.

In one embodiment, the lock enzyme carbohydrate can accumulate, and the key enzyme is targeted to the apoplast. The key enzyme can be targeted to the apoplast using, for example, the maize Gamma zein N-terminal signal sequence, which confers apoplast-specific targeting of proteins. The lock enzyme may be targeted to the amyloplast by, for example, fusion to the waxy amyloplast targeting peptide (Klosgen et al., Mol Gen Genet. 203: 237-244 (1986)) or to a starch granule. For example, the polynucleotide encoding the lock enzyme may be operably linked to a chloroplast (amyloplast) transit peptide (CTP) and a starch binding domain, e.g., from the waxy gene. Alternatively, the maize Brittle 1 transit peptide sequence (Bt1ts, Sullivan and Kaneko, Planta 196: 477-484 (1995)) can be used for amyloplast targeting. In other embodiments, the total carbohydrate content or sweetness or the endogenous carbohydrate content of the sink tissue is increased by targeting the carbohydrate-metabolizing enzyme to a sub-cellular compartment used for carbohydrate storage in the plant cells (e.g., vacuole or apoplasmic space).

A signal sequence such as the maize Gamma zein N-terminal signal sequence for targeting to the endoplasmic reticulum and secretion into the apoplast may be operably linked to a polynucleotide encoding the key enzyme in accordance with the present invention (Torrent et al., Plant Mol. Biol. 34:139 (1997)). Another signal sequence is the amino acid sequence SEKDEL (SEQ ID NO:7) for retaining polypeptides in the endoplasmic reticulum (Munro et al. Cell 48:899 (1987)).

Enhancers

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

Various intron sequences have been shown to enhance expression. For example, the introns of the maize *Adhl* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis et al., *Genes Develop.* 1: 1183-1200 (1987)). In the same experimental system, the intron from the maize bronze 1 gene had a similar effect in enhancing expression. Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression. Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (e.g. Gallie et al. *Nucl. Acids Res.* 15: 8693-8711 (1987); Skuzeski et al. *Plant Molec. Biol.* 15: 65-79 (1990)). Other leader sequences known in the art include but are not limited to: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein, O., Fuerst, T. R., and Moss, B. *PNAS USA* 86:6126-6130 (1989)); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison et al., *Virology* 154: 9-20 (1986)); MDMV leader (Maize Dwarf Mosaic Virus); *Virology* 154:9-20); human immunoglobulin heavy-chain binding protein (BiP) leader, (Macejak, D. G., and Samow, P., *Nature* 353: 90-94 (1991); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4), (Tobling, S. A., and Gehrke, L., *Nature* 325:622-625 (1987); tobacco mosaic virus leader (TMV), (Gallie, D. R. et al., *Molecular Biology of RNA*, pages 237-256 (1989); and Maize Chlorotic Mottle Virus leader (MCMV) (Lommel, S. A. et al., *Virology* 81:382-385 (1991). See also, Della-Cioppa et al., *Plant Physiology* 84:965-968 (1987).

Regulatory Sequences

The polynucleotides of the present invention, in addition to processing signals, may further include other regulatory sequences, as is known in the art. "Regulatory sequences" and "suitable regulatory sequences" each refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences include enhancers, promoters, translation leader sequences, introns, and polyadenylation signal sequences. They include natural and synthetic sequences as well as sequences that are a combination of synthetic and natural sequences.

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and correct mRNA polyadenylation. The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the DNA sequence of interest, the plant host, or any combination thereof). Appropriate transcriptional terminators are those that are known to function in plants and include the CAMV 35S terminator, the *tml* terminator, the nopaline synthase terminator and the pea *rbcS* E9 terminator. These can be used in both monocotyledons and dicotyledons. In addition, a gene's native transcription terminator may be used.

Selectable Markers

Generally, the expression cassette will comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Selectable markers may also be used in the present invention to allow for the selection of transformed plants and plant tissue, as is well-known in the art. One may desire to employ a selectable or screenable marker gene as, or in addition to, the expressible gene of interest. "Marker genes" are genes that impart a distinct phenotype to cells expressing the marker gene and thus allow such transformed cells to be distinguished from cells that do not have the marker. Such genes may encode either a selectable or screenable marker, depending on whether the marker confers a trait which one can select for by chemical means, i.e., through the use of a selective agent (e.g., a herbicide, antibiotic, or the like), or whether it is simply a trait that one can identify through observation or testing, i.e., by screening (e.g., the R-locus trait). Of course, many examples of suitable marker genes are known in the art and can be employed in the practice of the invention.

In one embodiment, both the lock and the key enzymes are expressed in the same plant, and the expression of the key enzyme is used as a selectable marker. In one example, the selection system is based on the expression of alpha-1,6-glucosidase in a plant accumulating isomaltulose. In such a system a means of breaking down isomaltulose into a substrate for fermentation is necessary, and may be provided in the form of sugarcane, sugarbeet, etc. plants engineered to express an alpha-1,6-glucosidase (isomaltulase, palatinase, etc.). Such a selectable marker system would be useful in screening for high level expression of alpha-1,6-glucosidase from the very earliest steps of plant transformation, this would be helpful in identifying integration events that are stable, highly expressed, and resistant to gene silencing. Also, this system could be used to select alpha-1,6-glucosidases with improved activity and in selecting for variants that increase protein or mRNA stability, localization to specific subcellular locations etc.

Also included within the terms selectable or screenable marker genes are also genes which encode a "secretable marker" whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected by their catalytic activity. Secretable proteins fall into a number of classes, including small, diffusible proteins detectable, e.g., by ELISA; small active enzymes detectable in extracellular solution (e.g., β -lactamase, phosphinothricin acetyltransferase); and proteins that are inserted or trapped in the cell wall (e.g., proteins that include a leader sequence such as that found in the expression unit of extension or tobacco PR-S).

With regard to selectable secretable markers, the use of a gene that encodes a protein that becomes sequestered in the cell wall, and which protein includes a unique epitope is also encompassed herein. Such a secreted antigen marker would ideally employ an epitope sequence that would provide low background in plant tissue, a promoter-leader sequence that would impart efficient expression and targeting across the plasma membrane, and would produce protein that is bound in the cell wall and yet accessible to antibodies. A normally secreted wall protein modified to include a unique epitope would satisfy all such requirements.

One example of a protein suitable for modification in this manner is extension, or hydroxyproline rich glycoprotein (HPRG). For example, the maize HPRG (Steifel et al., The

Plant Cell, 2:785 (1990)) molecule is well characterized in terms of molecular biology, expression and protein structure. However, any one of a variety of extensions and/or glycine-rich wall proteins (Keller et al., EMBO Journal, 8:1309 (1989)) could be modified by the addition of an antigenic site to create a screenable marker.

Possible selectable markers for use in connection with the present invention include, but are not limited to, a neo or nptII gene (Potrykus et al., Mol. Gen. Genet., 199:183 (1985)) which codes for kanamycin resistance and can be selected for using kanamycin, G418, and the like; a bar gene which confers resistance to the herbicide phosphinothricin; a gene which encodes an altered EPSP synthase protein (Hinchee et al., Biotech., 6:915 (1988)) thus conferring glyphosate resistance; a nitrilase gene such as bxn from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker et al., Science, 242:419 (1988)); a mutant acetolactate synthase gene (ALS) which confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (European Patent Application 154,204, 1985); a methotrexate-resistant DHFR gene (Thillet et al., J. Biol. Chem., 263:12500 (1988)); a dalapon dehalogenase gene that confers resistance to the herbicide dalapon; a phosphomannose isomerase (PMI) gene; a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan; the hph gene which confers resistance to the antibiotic hygromycin; or the mannose-6-phosphate isomerase gene (also referred to herein as the phosphomannose isomerase gene), which provides the ability to metabolize mannose (U.S. Pat. Nos. 5,767,378 and 5,994,629). One skilled in the art is capable of selecting a suitable selectable marker gene for use in the present invention.

An illustrative embodiment of a selectable marker gene capable of being used in systems to select transformants are the genes that encode the enzyme phosphinothricin acetyltransferase, such as the bar gene from *Streptomyces hygroscopicus* or the pat gene from *Streptomyces viridochromogenes*. The enzyme phosphinothricin acetyl transferase (PAT) inactivates the active ingredient in the herbicide bialaphos, phosphinothricin (PPT). PPT inhibits glutamine synthetase, (Murakami et al., Mol. Gen. Genet., 205:42 (1986); Twell et al., Plant Physiol., 91:1270 (1989)) causing rapid accumulation of ammonia and cell death. The success in using this selective system in conjunction with monocots was particularly surprising because of the major difficulties which have been reported in transformation of cereals (Potrykus, Trends Biotech., 7:269 (1989)).

Where one desires to employ a bialaphos resistance gene in the practice of the invention, a particularly useful gene for this purpose is the bar or pat genes obtainable from species of *Streptomyces* (e.g., ATCC No. 21,705). The cloning of the bar gene has been described (Murakami et al., Mol. Gen. Genet., 205:42 (1986); Thompson et al., EMBO Journal, 6:2519 (1987)) as has the use of the bar gene in the context of plants other than monocots (De Block et al., EMBO Journal, 6:2513 (1987); De Block et al., Plant Physiol., 91:694 (1989)).

Screenable markers that may be employed include, but are not limited to, a β -glucuronidase or uidA gene (GUS) which encodes an enzyme for which various chromogenic substrates are known; an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta et al., in Chromosome Structure and Function, pp. 263-282 (1988)); a β -lactamase gene (Sutcliffe, PNAS USA, 75:3737 (1978)), which encodes an enzyme for which various chromogenic substrates are known (e.g., PADAC, a chromogenic cephalosporin); a xyle gene (Zukowsky et al., PNAS USA, 80:1101 (1983)) which encodes a catechol dioxygenase that can convert chromoge-

nic catechols; a tyrosinase gene (Katz et al., J. Gen. Microbiol., 129:2703 (1983)) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to form the easily detectable compound melanin; a β -galactosidase gene, which encodes an enzyme for which there are chromogenic substrates; a luciferase (lux) gene (Ow et al., Science, 234:856 (1986)), which allows for bioluminescence detection; or an aequorin gene (Prasher et al., Biochem. Biophys. Res. Comm., 126:1259 (1985)), which may be employed in calcium-sensitive bioluminescence detection, or a green fluorescent protein gene (Niedz et al., Plant Cell Reports, 14: 403 (1995)).

Genes from the maize R gene complex are contemplated to be particularly useful as screenable markers. The R gene complex in maize encodes a protein that acts to regulate the production of anthocyanin pigments in most seed and plant tissue. A gene from the R gene complex is suitable for maize transformation, because the expression of this gene in transformed cells does not harm the cells. Thus, an R gene introduced into such cells will cause the expression of a red pigment and, if stably incorporated, can be visually scored as a red sector. If a maize line carries dominant alleles for genes encoding the enzymatic intermediates in the anthocyanin biosynthetic pathway (C2, A1, A2, Bz1 and Bz2), but carries a recessive allele at the R locus, transformation of any cell from that line with R will result in red pigment formation. Exemplary lines include Wisconsin 22 which contains the rg-Stadler allele and TR112, a K55 derivative which is r-g, b, P1. Alternatively any genotype of maize can be utilized if the C1 and R alleles are introduced together. A further screenable marker contemplated for use in the present invention is firefly luciferase, encoded by the lux gene. The presence of the lux gene in transformed cells may be detected using, for example, X-ray film, scintillation counting, fluorescent spectrophotometry, low-light video cameras, photon counting cameras or multiwell luminometry. It is also envisioned that this system may be developed for populational screening for bioluminescence, such as on tissue culture plates, or even for whole plant screening.

Additional Agronomic Traits

The plants disclosed herein may further exhibit one or more agronomic traits that primarily are of benefit to a seed company, a grower, or a grain processor, for example, herbicide resistance, virus resistance, bacterial pathogen resistance, insect resistance, nematode resistance, and fungal resistance. See, e.g., U.S. Pat. Nos. 5,569,823; 5,304,730; 5,495,071; 6,329,504; and 6,337,431. Such trait may also be one that increases plant vigor or yield (including traits that allow a plant to grow at different temperatures, soil conditions and levels of sunlight and precipitation), or one that allows identification of a plant exhibiting a trait of interest (e.g., selectable marker gene, seed coat color, etc.). Various traits of interest, as well as methods for introducing these traits into a plant, are described, for example, in U.S. Pat. Nos. 5,569,823; 5,304,730; 5,495,071; 6,329,504; 6,337,431; 5,767,366; 5,928,937; 4,761,373; 5,013,659; 4,975,374; 5,162,602; 4,940,835; 4,769,061; 5,554,798; 5,879,903; 5,276,268; 5,561,236; 4,810,648; and 6,084,155; in European application No. 0 242 246; in U.S. Patent Application No. 20010016956; and on the worldwide web at www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/. Plant Transformation

Once a nucleic acid sequence encoding the lock and/or key enzyme has been cloned into an expression system, it is transformed into a plant cell. The word "plant" refers to any plant, particularly to seed plant, and "plant cell" is a structural and physiological unit of the plant, which comprises a cell

wall but may also refer to a protoplast. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, or a plant organ. The term "transformation" refers to the transfer of a nucleic acid fragment into the genome of a host cell, resulting in genetically stable inheritance. Host cells containing the transformed nucleic acid fragments are referred to as "transgenic" cells, and organisms comprising transgenic cells are referred to as "transgenic organisms."

Examples of methods of transformation of plants and plant cells include *Agrobacterium*-mediated transformation (De Blaere et al., 1987) and particle bombardment technology (Klein et al. 1987; U.S. Pat. No. 4,945,050). Whole plants may be regenerated from transgenic cells by methods well known to the skilled artisan (see, for example, Fromm et al., 1990).

The expression cassettes of the present invention can be introduced into the plant cell in a number of art-recognized ways. The term "introducing" in the context of a polynucleotide, for example, a nucleotide encoding an enzyme disclosed herein, is intended to mean presenting to the plant the polynucleotide in such a manner that the polynucleotide gains access to the interior of a cell of the plant. Where more than one polynucleotide is to be introduced, these polynucleotides can be assembled as part of a single nucleotide construct, or as separate nucleotide constructs, and can be located on the same or different transformation vectors.

Accordingly, these polynucleotides can be introduced into the host cell of interest in a single transformation event, in separate transformation events, or, for example, in plants, as part of a breeding protocol. The methods of the invention do not depend on a particular method for introducing one or more polynucleotides into a plant, only that the polynucleotide(s) gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotides into plants are known in the art including, but not limited to, transient transformation methods, stable transformation methods, and virus-mediated methods.

"Transient transformation" in the context of a polynucleotide is intended to mean that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant.

By "stably introducing" or "stably introduced" in the context of a polynucleotide introduced into a plant is intended the introduced polynucleotide is stably incorporated into the plant genome, and thus the plant is stably transformed with the polynucleotide.

"Stable transformation" or "stably transformed" is intended to mean that a polynucleotide, for example, a nucleotide construct described herein, introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations.

Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation arts, and the genes pertinent to this invention can be used in conjunction with any such vectors. The selection of vector will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred as discussed elsewhere herein.

Methods for regeneration of transformed plants are well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of foreign DNA, as well as direct DNA uptake, liposomes, electroporation, microinjection, and microprojectiles. In addition, bacteria from the genus *Agrobacterium* can be utilized to transform plant cells. Below are

descriptions of representative techniques for transforming both dicotyledonous and monocotyledonous plants, as well as a representative plastid transformation technique.

Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, Nucl. Acids Res. (1984)). For the construction of vectors useful in *Agrobacterium* transformation, see, for example, US Patent Application Publication No. 2006/0260011, herein incorporated by reference.

Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can also be utilized. Transformation techniques that do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (e.g. PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. For the construction of such vectors, see, for example, US Application No. 20060260011, herein incorporated by reference.

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques that do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This method can be accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are described by Paszkowski et al., EMBO J. 3: 2717-2722 (1984), Potrykus et al., Mol. Gen. Genet. 199: 169-177 (1985), Reich et al., Biotechnology 4: 1001-1004 (1986), and Klein et al., Nature 327: 70-73 (1987). In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

Agrobacterium-mediated transformation is a preferred technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. *Agrobacterium* transformation typically involves the transfer of the binary vector carrying the foreign DNA of interest to an appropriate *Agrobacterium* strain which may depend of the complement of vir genes carried by the host *Agrobacterium* strain either on a co-resident Ti plasmid or chromosomally (Uknes et al. Plant Cell 5: 159-169 (1993)). The transfer of the recombinant binary vector to *Agrobacterium* is accomplished by a triparental mating procedure using *E. coli* carrying the recombinant binary vector, a helper *E. coli* strain which carries a plasmid that is able to mobilize the recombinant binary vector to the target *Agrobacterium* strain. Alternatively, the recombinant binary vector can be transferred to *Agrobacterium* by DNA transformation (Hofgen & Willmitzer, Nucl. Acids Res. 16: 9877 (1988)).

Transformation of the target plant species by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.

Another approach to transforming plant cells with a gene involves propelling inert or biologically active particles at plant tissues and cells. This technique is disclosed in U.S. Pat. Nos. 4,945,050, 5,036,006, and 5,100,792. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and afford incorporation within the

interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the desired gene. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried yeast cells, dried bacterium or a bacteriophage, each containing DNA sought to be introduced) can also be propelled into plant cell tissue.

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, and particle bombardment into callus tissue. Transformations can be undertaken with a single DNA species or multiple DNA species (i.e. co-transformation) and both of these techniques are suitable for use with this invention. Co-transformation may have the advantage of avoiding complete vector construction and of generating transgenic plants with unlinked loci for the gene of interest and the selectable marker, enabling the removal of the selectable marker in subsequent generations, should this be regarded desirable.

Patent Applications EP 0 292 435, EP 0 392 225, and WO 93/07278 describe techniques for the preparation of callus and protoplasts from an elite inbred line of maize, transformation of protoplasts using PEG or electroporation, and the regeneration of maize plants from transformed protoplasts. Gordon-Kamm et al. (Plant Cell 2: 603-618 (1990)) and Fromm et al. (Biotechnology 8: 833-839 (1990)) have published techniques for transformation of A188-derived maize line using particle bombardment. Furthermore, WO 93/07278 and Kozziel et al. (Biotechnology 11: 194-200 (1993)) describe techniques for the transformation of elite inbred lines of maize by particle bombardment. This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days after pollination and a PDS-1000He Biolistics device for bombardment.

The plants obtained via transformation with a nucleic acid sequence of the present invention can be any of a wide variety of plant species, including those of monocots and dicots; however, the plants used in the method of the invention are preferably selected from the list of agronomically important target crops set forth supra. The expression of a gene of the present invention in combination with other characteristics important for production and quality can be incorporated into plant lines through breeding. Breeding approaches and techniques are known in the art. See, for example, Welsh J. R., Fundamentals of Plant Genetics and Breeding, John Wiley & Sons, NY (1981); Crop Breeding, Wood D. R. (Ed.) American Society of Agronomy Madison, Wis. (1983); Mayo O., The Theory of Plant Breeding, Second Edition, Clarendon Press, Oxford (1987); Singh, D. P., Breeding for Resistance to Diseases and Insect Pests, Springer-Verlag, NY (1986); and Wricke and Weber, Quantitative Genetics and Selection Plant Breeding, Walter de Gruyter and Co., Berlin (1986).

The genetic properties engineered into the transgenic seeds and plants described above are passed on by sexual reproduction or vegetative growth and can thus be maintained and propagated in progeny plants. Generally, maintenance and propagation make use of known agricultural methods developed to fit specific purposes such as tilling, sowing or harvesting.

The lock and/or key enzymes disclosed herein may also be incorporated into or maintained in plant lines through breeding or through common genetic engineering technologies. Breeding approaches and techniques are known in the art. See, for example, Welsh J. R., Fundamentals of Plant Genetics and Breeding, John Wiley & Sons, NY (1981); Crop Breeding, Wood D. R. (Ed.) American Society of Agronomy

Madison, Wis. (1983); Mayo O., The Theory of Plant Breeding, Second Edition, Clarendon Press, Oxford (1987); Singh, D. P., Breeding for Resistance to Diseases and Insect Pests, Springer-Verlag, NY (1986); and Wricke and Weber, Quantitative Genetics and Selection Plant Breeding, Walter de Gruyter and Co., Berlin (1986).

The relevant techniques are well known in the art and include but are not limited to hybridization, inbreeding, back-cross breeding, multi-line breeding, dihaploid inbreeding, variety blend, interspecific hybridization, aneuploid techniques, etc. Hybridization techniques also include the sterilization of plants to yield male or female sterile plants by mechanical, genetic (including transgenic), chemical, or biochemical means.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by J. Sambrook, E. F. Fritsch and T. Maniatis, Molecular Cloning: A Laboratory manual, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989) and by T. J. Silhavy, M. L. Berman, and L. W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

EXAMPLE 1

Enzymes that can Produce Locked Sugars

1A: Bacterial Expression System of His-Tagged Enzymes

Selected genes coding for specific enzymes were cloned into an *Escherichia coli* expression vector, pET24b (Novagen), using restriction sites that place the coding sequence in-frame downstream of an inducible T7lac promoter. Polynucleotide sequences coding for specific enzymes were generated by back translating the polypeptide sequence of the enzyme using the codon preference for *E. coli*. The expression plasmids were introduced into an *E. coli* expression strain, BL21 Star (DE3) (Invitrogen). Recombinant *E. coli* isolates containing the modified pET24b expression vector were selected on standard LB agar containing 50 ug/mL kanamycin.

Recombinant *E. coli* isolates were grown with shaking at 37 degrees C. for 8 hours to overnight in 20 mL of LB media containing 50 ug/mL kanamycin. The 20 mL of *E. coli* culture was transferred to 1 L of autoinduction media (9.57 g tryptone, 4.8 g yeast extract, 2 mL of 1 M MgSO₄, 1 mL of 1000x trace metals, 20 mL of 50x5052, 20 mL of 50xM) (1000x trace metals: 36 mL sterile water, 50 mL of 0.1M FeCl₃ in 0.12M HCl, 2 mL of 1M CaCl₂, 1 mL of 1M MnCl₂ 4 H₂O, 1 mL of 1M ZnSO₄ 7 H₂O, 1 mL of 0.2M CoCl₂ 6 H₂O, 2 mL of 0.1M CuCl₂ 2 H₂O, 1 mL of 0.2M NiCl₂ 6 H₂O, 2 mL of 0.1M Na₂MoO₄ 2 H₂O, 2 mL of 0.1M H₃BO₃) (50x5052: 25 g glycerol, 73 mL H₂O, 2.5 g glucose 10 g alpha-lactose monohydrate) (50xM: 80 mL H₂O, 17.75 g Na₂HPO₄, 17.0 g KH₂PO₄, 13.4 g NH₄Cl, 3.55 g Na₂SO₄) with 25 ug/mL kanamycin and grown with shaking at 28 degrees C. overnight. The *E. coli* cells were harvested out of the autoinduction media by centrifugation at 10,000xg for 15 minutes and the collected cells were frozen at -80 degrees C.

1B: Sucrose Isomerase (E.C. 5.4.99.11)

The amino acid sequence for a sucrose isomerase expressed by *Erwinia carotovora* has been listed in GeneBank under the accession number YP049947 (SEQ ID NO: 14). The amino acid sequence of this sucrose isomerase was back translated into a polynucleotide coding sequence using the codon preference of *E. coli*. The polynucleotide sequence was generated by gene synthesis (GeneArt) and cloned into the expression vector pET24b (Novagen) using restriction sites that place the coding sequence in-frame downstream of an inducible T7lac promoter. This expression plasmid was introduced into an *E. coli* expression strain, BL21, harboring λ DE3 lysogen. After growing for 3 hours in LB media containing 50 microgram/microliter kanamycin, the cells were induced to produce the *E. carotovora* sucrose isomerase enzyme with IPTG at a final concentration of 1 mM. The *E. coli* cells were harvested 3 hours after induction by centrifugation at 10,000 \times g for 10 min and the supernatant was removed. Cells were lysed by resuspending the cell pellet in BugBuster reagent (Novagen) containing lysozyme (1KU/1 mL BugBuster) and benzonase (25 units/1 mL BugBuster) followed by incubation for 10 min on a shaking platform. Insoluble debris was removed by centrifugation at 16,000 \times g for 20 min at 4 degrees C. Supernatant containing total soluble protein and the recombinant enzyme was transferred to a fresh 1.5 mL Eppendorf tube and aliquots were stored at 4 degrees C. and -20 degrees C. for further characterization.

Sucrose isomerase enzyme activity was assayed by combining the enzyme with the substrate, sucrose, and measuring the production of isomaltulose and trehalulose. The total soluble protein extract from the recombinant *E. coli* was assayed for sucrose isomerase activity by incubating 10 microliters of supernatant *E. coli* lysate, as described above, with 90 microliters of 292 mM sucrose 50 mM sodium phosphate buffer (pH 6.0) at 30 degrees C. for 20 hours. The reaction product was screened for the presence of isomaltulose and trehalulose by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC).

TLC was performed by spotting 3 microliters of the supernatants of the growth media onto AL SIL G silica gel plates (Whatman) and developed twice in a solvent consisting of 3 parts ethylacetate: 3 parts acetic acid: 1 part distilled water. After drying, the plates were sprayed with a dye mixture consisting of 4 milliliters aniline, 4 g diphenylamine, 200 milliliters acetone, and 30 milliliters 80% phosphoric acid. Isomaltulose and trehalulose were distinguished from other sugars, such as sucrose, by their relative mobility and by the distinct colors produced when they reacted with aniline dye. Greenish yellow indicates the presence of isomaltulose, red indicates the presence of trehalulose, and brown/black indicates the presence of sucrose. The monosaccharides, glucose and fructose, produced by hydrolysis of sucrose were blue or red-orange respectively.

Identification of the sugars present in each lane of the developed TLC plate was possible by comparing both the relative mobility of the sugars present in the samples and the staining color with aniline dye to the relative mobility and staining color of sugar standards. The reaction product of sucrose isomerase incubated with sucrose as described above was three colored bands. The highest mobility band had a purple color and migrated with the same mobility as both glucose and fructose standards blue and red colored respectively and is therefore interpreted to be a mixture of co migrating glucose and fructose released by hydrolysis of one of the disaccharides: sucrose, isomaltulose, or trehalulose. The middle band corresponded with the isomaltulose standard in

both coloration and relative mobility and is therefore identified as isomaltulose. The slowest migrating band had a red coloration and migrated slower than either the isomaltulose, or sucrose standards. The relative mobility of this sugar band corresponds well with published reports on the migration of trehalulose in similar TLC assays (Cho et al. Biotechnology Letters (2007) 29:453-458; an isomaltulose-producing microorganism isolated from traditional Korean food.) Therefore this sugar band was concluded to be trehalulose. No trehalulose standard was available at the time of the TLC assay, however, subsequent HPLC (Dionex) analysis of sucrose isomerase reaction products and standards obtained later indicate that this band was definitely trehalulose. Also, it is important to note that the reaction product 6 did not contain any sucrose which has a higher relative mobility than isomaltulose and trehalulose and slower mobility than the monosaccharides glucose and fructose. The absence of sucrose was expected due to the complete conversion of sucrose into isomaltulose and trehalulose due to the activity of the sucrose isomerase enzyme.

Alternatively, supernatants were screened by HPCL using 16 mM NaOH to separate sucrose isomerase reaction products followed by a linear gradient from 10 to 40 min using 200 mM NaOH at 1 ml/min on a Dionex DX-600 system with ED50 electrochemical detector (Dionex Co.).

His-Tagged Sucrose Isomerase (SEQ ID NO: 14)

Recombinant BL21[DE3] cell pellets expressing his-tagged sucrose isomerase (SEQ ID NO: 14) were generated essentially as described in Example 1A. The recombinant BL21 cell pellets were brought up to a volume of 40 mL in extraction buffer (50 mM sodium phosphate, 500 mM NaCl, 10 mM Imidazole, pH 8 containing protease inhibitors (Roche Complete EDTA-free protease inhibitor tablets)). Cells were lysed by 2 passages through a FRENCH Press (Thermo IEC). Cell lysate was centrifuged for 30 minutes at 10,000 \times g at 4 degrees C. Supernatant was filtered using 0.45 micron vacuum filter devices (Millipore) to generate a clarified lysate. A HisTrap FF 5 ml column (GE Healthcare) was equilibrated with extraction buffer. The clarified lysate was loaded onto the equilibrated column at 5 mL/min. Bound his-tagged sucrose isomerase was eluted in a linear imidazole gradient from 50 mM sodium phosphate, 500 mM NaCl, 10 mM Imidazole, pH 8 to 50 mM sodium phosphate, 500 mM NaCl, 200 mM Imidazole over 100 mL. Fractions containing the enzyme were collected and diluted in 50 mM Tris-HCl, pH 8. Diluted sample was loaded onto a 5 mL HiTrap Q HP anion exchange column (GE Healthcare). Bound proteins were eluted from the column by running a linear NaCl gradient from 50 mM Tris-HCl, pH 8 to 50 mM Tris-HCl, 500 mM NaCl, pH 8 over 100 mL. Active sucrose isomerase was detected in the flow through and fractions that eluted at approximately 100 mM NaCl. These fractions were pooled and concentrated to a final protein concentration of 0.8 mg/mL. Samples were aliquoted and stored at -80 degrees C.

Sucrose isomerase enzyme activity was measured in the samples by combining 6 ug/mL his-tagged sucrose isomerase, 70 mM 0.1 M Citrate-phosphate buffer, pH 6 and 584 mM sucrose at 30 degrees C. for 2 hours. Sample was analyzed by Dionex essentially as described in Example 1G. Table 1 outlines the sucrose isomerase activity detected in recombinant *E. coli* cells expressing sucrose isomerase (SEQ ID NO: 14). Activity is demonstrated by the accumulation of the locked sugars trehalulose and isomaltulose.

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TABLE 1

Sucrose isomerase (SEQ ID NO: 14) activity measured using sucrose as the substrate after 2 hr.					
Time	Glucose (mM)	Fructose (mM)	Sucrose (mM)	Trehalulose (mM)	Isomaltulose (mM)
Sucrose isomerase	5.98	4.97	0.61	227.96	248.45
Negative control	0	0	512	0	0

1C: Dextranucrase Enzyme (E.C. 2.4.1.5)

Dextranucrases (E.C. 2.4.1.5) are glucosyl transferase enzymes capable of transferring glucose from a sucrose molecule to form glucose homopolymers known as dextrans. This type of enzymatic reaction is an example of transglycosylation. The dextran is composed of mainly 1,6 alpha D glucose linkages of varying length. The dextran can also contain a variety of 1,4 alpha D glucose linkages which form branch points in the dextran molecule. These branching points have a direct impact on the physiochemical properties (such as solubility) of the dextran molecules. The polynucleotide sequence coding for a dextranucrase enzyme will be generated that uses the codon preference for *E. coli*. This polynucleotide sequence will be synthesized, cloned into an expression vector and expressed in *E. coli* as described in Example 1A.

Dextranucrase enzyme activity will be monitored using a colorimetric assay to detect the rate of fructose release from sucrose (Kobayashi, M et al. (1980) *Biochimica et Biophysica Acta* vol 614, pp 46-62). Dextran accumulation will be monitored using methods similar to those described in Zhang, S., et al. (2007) *Transgenic Res.* 16:467-478 in combination with HPLC techniques such as size exclusion chromatography. Dextranucrase enzyme activity assays will be validated by comparing dextranucrase activity recovered from recombinant *E. coli* with commercially available dextranucrase enzyme.

Dextranucrase activity will be measured using sugarcane juice as the source of sucrose. Selected *E. coli* expressed dextranucrases will be incubated in a similar fashion as described above, however sucrose will be replaced with sugarcane juice as the substrate. These experiments will be designed to test the ability of the expressed enzymes to produce dextrans from sucrose in the presence of other proteins and unknown compounds found in sugarcane juice.

A mutant dextranucrase has been characterized by Hellmuth et al. *Biochemistry* 47: 6678-6684 (2008) which alters the activity of the enzyme such that it can catalyze the conversion of sucrose to isomaltulose or leucrose. This dextranucrase variant has leucrose synthase activity due to the ability of the variant enzyme to catalyze the conversion of sucrose to leucrose.

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Analysis of His-Tagged Dextranucrase with Leucrose Synthase Activity (SEQ ID NO: 29).

Recombinant BL21[DE3] cell expressing a His-tagged dextranucrase with leucrose synthase activity (SEQ ID NO: 29) was generated essentially as described in Example 1A. Frozen cell pellets were brought up to a volume of 30-40 mL in extraction buffer (50 mM sodium phosphate, 500 mM NaCl, 10 mM Imidazole, pH 7.2 containing protease inhibitors (Roche Complete EDTA-free protease inhibitor tablets)). Cells were lysed by 2 passages through a FRENCH Press (Thermo IEC). Cell lysates were centrifuged for 30 minutes at 10,000×g at 4 degrees C. Supernatants were filtered using 0.45 micron vacuum filter devices (Millipore). A HisTrap FF 5 ml column (GE Healthcare) was equilibrated with extraction buffer and the clarified lysates were loaded at 5 mL/min. Bound his-tagged enzymes were eluted in 50 mM sodium phosphate, 500 mM NaCl, containing 300 mM Imidazole, pH 7.2. All samples were buffer exchanged into 50 mM HEPES, 50 mM NaCl, pH 7 using a HiPrep 26/10 desalting column (GE Healthcare). 50% Glycerol was added to such that the final buffer was 40 mM HEPES, 40 mM NaCl, 10% glycerol, pH 7. Protein concentrations were estimated by Bradford assay. Samples were stored at -80 degrees C.

As a negative control, BL21[DE3] cell pellets expressing the empty pET24b vector were processed as above except for elution from HisTrap was in 50 mM sodium phosphate, 500 mM NaCl, containing 500 mM Imidazole, pH 7.2.

His-tagged dextranucrase with leucrose synthase activity was diluted to 0.1 mg/mL in 40 mM HEPES, 40 mM NaCl, 10% glycerol, pH 7.2-100 uL reactions were set up for the leucrose synthase and the negative control with the following conditions:

	#1	#2
Sample (0.1 mg/ml)	10	10
Buffer (200 mM Sorensen's Buffer + 500 mM CaCl ₂ , pH 7)	60.8	60.8
2M Sucrose	14.6	14.6
2M Fructose	0	14.6
Water	14.6	0
Total Reaction Volume	100	100

Volumes in column #1 and #2 are in microliters

Table 2 outlines data demonstrating that his-tagged dextranucrase (SEQ ID NO: 29) with leucrose synthase activity is enzymatically active and converts sucrose to leucrose and isomaltose. Dextranucrase enzymes catalyze the conversion of sucrose to locked sugars through a transglycosylation reaction. Table 2, comparing sample 1 and sample 2, demonstrates that dextranucrase with leucrose synthase activity has altered specificity toward producing leucrose versus isomaltose dependent on the addition of fructose as a secondary substrate.

TABLE 2

Dionex analysis of carbohydrate products from microbially expressed His-tagged dextranucrase with leucrose synthase activity. Enzyme activity indicated by the change in percent sugar determined by comparing samples collected at time 0 and time 24 hours.						
Sample set up	Glucose (% total sugar)	Fructose (% total sugar)	Sucrose (% total sugar)	Isomaltose (% total sugar)	Isomaltulose (% total sugar)	Leucrose (% total sugar)
1	8.99	20.55	-37.46	3.16	0.66	4.09
2	1.40	-0.29	-6.57	0.12	0.57	4.77

TABLE 2-continued

Dionex analysis of carbohydrate products from microbially expressed His-tagged dextranucrase with leucrose synthase activity. Enzyme activity indicated by the change in percent sugar determined by comparing samples collected at time 0 and time 24 hours.						
Sample set up	Glucose (% total sugar)	Fructose (% total sugar)	Sucrose (% total sugar)	Isomaltose (% total sugar)	Isomaltulose (% total sugar)	Leucrose (% total sugar)
1 (Negative control)	0.08	0.14	-0.22	0	0	0
2 (Negative control)	-0.01	0.63	-0.62	0	0	0

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control contains bacterial fractions collected as described in Example 1A from cells containing an empty pET24 vector.

1D: Levan Sucrase, Fructosyl Transferase (E.C. 2.4.1.10, E.C. 2.4.1.99, E.C. 2.4.1.100)

Sucrose:sucrose fructosyltransferase (SST) (EC 2.4.1.99), 1,2- β -fructan 1-fructosyltransferase (FFT) (EC 2.4.1.100), and levan sucrase (EC 2.4.1.10) are enzymes within the larger class of fructosyl transferases. The fructosyl transferase enzymes catalyze the formation of fructans composed of fructose linked by $\beta(2\rightarrow1)$ and/or $\beta(2\rightarrow6)$ glucoside bonds. Fructosyl transferases may be identified and isolated from plant, bacterial, or fungal sources. These enzymes may be expressed in plants to accumulate fructans as storage carbohydrates. Accumulation of this polysaccharide (fructan) in sugarcane or other plants may allow the accumulation of excess carbohydrates.

The polynucleotide sequence coding for a fructosyltransferase enzyme will be generated that uses the codon preference for *E. coli*. This polynucleotide sequence will be synthesized, cloned into an expression vector and expressed in *E. coli* essentially as described in Example 1A.

Fructosyl transferase activity will be estimated by TLC and HPLC similar to the procedures described above for sucrose isomerase and the Dionex analysis described in Example 1B. Modifications to the protocol in order to increase the sensitivity for fructans may include development in a solution of propanol:butanol:water (12:3:4) and the use of a urea-phosphoric acid dye mixture (Wise et al., 1955, Anal Chem 27:33-36). Long polymers of fructose have low mobility in the TLC assay and will remain in the location where they are spotted on the silica gel plate. Hydrolysis of fructans to fructose by HCl solution will allow specific identification of fructose using the aniline dye described above. Alternatively a fructanase enzyme may be used to hydrolyze fructans to fructose. This technique will be useful in determining that large polymers are indeed fructans as only fructans would be hydrolyzed by a fructanase enzyme.

Fructose, as the sweetest naturally occurring sugar, also has value as a sweetener in high fructose syrups such as high fructose corn syrup. Plants engineered to produce fructans as a locked sugar may be used as a source of fructans which, after hydrolysis by a fructanase enzyme, produce a solution with a high fructose concentration. In such plants the yield of fructan may be increased by expressing an additional enzyme glucose isomerase to catalyze the conversion of glucose to fructose. The glucose isomerase (invertase) could be expressed in maize endosperm, or expressed in microbes. The purified enzyme could be used to produce fructans, glucans and alternans.

Sweeter plant products can be generated by expressing in plants a combination of enzymes that first allow for the accu-

mulation of fructans in the plant and then convert the fructans directly or indirectly to fructose. Expressing invertase (glucose isomerase) in plants accumulating fructans will lead to a higher sweetness index in the plant.

Endogenous sucrose synthase activity in the endosperm will create additional sucrose which may be used as a substrate for further fructan synthesis.

1E: Alternansucrase

Alternan is a polysaccharide consisting of glucosyl residues linked by alternate $\alpha(1-3)/\alpha(1-6)$ bonds. This polymer is highly soluble and has very low viscosity. Accumulation of this polysaccharide in sugarcane or other plants may allow the accumulation of excess carbohydrates. Alternansucrase is an enzyme which catalyzes the conversion of sucrose to alternan.

Alternansucrase is encoded by the Asr gene of *Leuconostoc mesenteroides* NRRL B-1355, 1498, and 1501 (Jeannes et al. Am Chem Soc 76:5041-5052, 1954). The Asr gene may be synthesized, cloned into an expression vector and expressed in *E. coli* essentially as described in Example 1A.

Alternansucrase activity may be detected by enzyme-linked immunosorbent assay (ELISA) as described by Kok-Jacor et al. J. Plant Physiol 160: 765-777 (2005) Alternans can be hydrolyzed to form fermentable sugars by the activity of a $\alpha(1-6)$ -glucosidase or $\alpha(1-3)$ -glucosidase or a combination of the two enzymes.

1F: Amylosucrase (E.C. 2.4.1.4)

Amylose or starch, is a polysaccharide consisting of glucosyl residues linked by $\alpha(1-4)$ bonds and is the primary carbohydrate storage compound found in most plants. Producing starch in plants that use sucrose as their primary carbohydrate storage compound, such as sugarcane, may permit the accumulation of starch which would behave as a locked sugar.

Neisseria polysacharea produces an amylosucrase enzyme (GenBank Accession number Q9ZEU2) which catalyzes the conversion of sucrose to a linear $\alpha(1-4)$ -linked glucan. For the purpose of producing starch in a transgenic plant, it may be advantageous to target the amylosucrase enzyme in the plant to subcellular compartments that have high concentrations of sucrose, such as the vacuole of sugarcane. Another target may be the vacuole of the maize endosperm. Targeting an enzyme capable of synthesizing starch from sucrose to the vacuole of maize endosperm cells may permit the accumulation of more starch in the maize endosperm as naturally occurring enzymes do not produce starch in the vacuoles of maize endosperm cells. Targeting such an enzyme to endosperm vacuoles may be expected to create up to 10% more starch because of starch accumulation in a subcellular

compartment that normally does not accumulate starch. Alternatively targeting to the apoplast is another way to achieve conversion of sucrose into locked sugars such as starch or isomaltulose. In plants such as maize, sucrose accumulates in the leaf and is transported to the ear during grain filling which provides a carbon sink. Table 3 outlines the sugar content of maize tissue with and without removal of the ear. Note that when the ear is removed, excess sugar accumulates in the leaf tissue.

TABLE 3

Sugar content of maize with and without ears.		
Sugar, mg/mL	Earless maize	Maize with Ear
Sucrose	7.42	2.6
Glucose	1.34	1.05
Fructose	1.32	0.95
Total, mg/mL	10.08	4.6

A codon optimized polynucleotide sequence coding for the *N. polysacharea* amylosucrase enzyme may be synthesized, cloned into an expression vector and expressed in *E. coli* essentially as described in Example 1A.

His-Tagged Amylosucrase

Recombinant BL21 cells expressing an amylosucrase will be generated essentially as described in Example 1A. Frozen BL21[DE3] cell pellets expressing amylosucrase will be recovered from a 30 mL overnight culture in autoinduction media and will be resuspended in 3 mL BugBuster HT (Novagen) containing Complete EDTA-free protease inhibitors (Roche). Samples will be incubated at room temperature for 10 minutes with occasional mixing to lyse cells. Cell lysate will be centrifuged at 10,000×g for 10 minutes at 4 degrees C. 10 uL of supernatant will be incubated in a 500 uL reaction containing 1×PBS and 100 mM sucrose overnight at 30 degrees C. The presence of a visible white precipitate indicates amylosucrase activity. Determination that this precipitate is starch can be done by washing the precipitate in 80% ethanol several times, followed by solubilization in DMSO and gel permeation chromatography. Susceptibility to digestion by amylase enzyme would further demonstrate the precipitate is composed of starch.

1G: Dionex HPAEC Analysis of Carbohydrates

Carbohydrate separation and detection was analyzed utilizing a Dionex IC3000 system with a Dionex AS autosampler, a Dionex DC detection compartment (pulsed amperometric detection (PAD) using a disposable Dionex carbohydrate certified gold surface electrode), and a Dionex SP pump system. For high resolution separation, one Carbo-pac PA1 4×50 mM Guard Column followed by two Carbo-pac PA1 4×250 mM analytical columns were used for all analysis. The electrode potentials were set to the carbohydrates standard quad with AgCl reference electrode as specified by Dionex Corporation. The eluent system utilized an isocratic mobile phase consisting of 100 mM NaOH and 2 mM NaOAc with a 38 min run time. Peak identification was based on standard retention times of glucose, fructose, sucrose (Sigma), leucrose (Carbosynth), isomaltulose (Fischer) and trehalulose. Peak analysis utilized Chromeleon version 6.80 software (Dionex Corp., Sunnyvale, Calif.).

EXAMPLE 2

Enzymes that Unlock Locked Sugars

2A: Fructanase (EC 3.2.1.80, E.C. 3.2.1.7)

Fructanases are fructosydases which catalyze the hydrolysis of fructosidic linkages in fructans to break the fructan down into simpler sugar molecules. Fructans can be hydrolyzed to fermentable sugars through the catalytic activity of fructanases. For Example, the fructanase 2,1-β-D-fructan fructanohydrolase [EC 3.2.1.7] can hydrolyze fructan polymers into fructose monosaccharides which can be fermented to form ethanol.

A codon optimized polynucleotide sequence coding for a fructanase enzyme may be synthesized, cloned into an expression vector and expressed in *E. coli* essentially as described in Example 1A.

Fructanase activity may be estimated by incubating a fructanase enzyme with a solution of fructan. Hydrolysis of fructan by the fructanase will release the monosaccharide fructose which may be detected by TLC or HPLC as described above for sucrose isomerase (Example 1B).

2B: Glucosidase

Gene sequences for alpha-1,6-glucosidases were identified using BLAST to search the NCBI database for genes homologous to a known alpha-1,6-glucosidase. The polypeptide sequences (SEQ ID NOs: 1-6) were back translated (using Vector NTI program) into polynucleotide sequences using the codon preference of *E. coli*. The *E. coli* codon optimized polynucleotide sequences were synthesized by GeneArt and expressed in *E. coli* essentially as described in Example 1B.

Alpha-1,6-glucosidase activity was assayed by measuring the production of glucose from hydrolysis of the alpha-1,6-glucoside bond of isomaltulose. 13 microliters of crude *E. coli* extract was added to 37 microliters of isomaltulose reaction buffer (100 mM isomaltulose and 30 mM HEPES (pH 7.5)) at 30 degrees, 40 degrees, 50 degrees, 60 degrees, 70 degrees, or 80 degrees C. depending on the enzyme; for 10 minutes, 20 minutes, 30 minutes, or 40 minutes. 20 microliters of the reaction product was added to a 96 well microplate, then 250 microliters of glucose oxidase reagent (Pointe Scientific) was added and the mixture was incubated at 37 degrees C. for 10 minutes. After this incubation, the Absorbance at 500 nm was read using a SpectraMax plus 384. Sample absorbance was compared with the absorbance at 500 nm of controls which were 13 microliters each of a set of glucose standards that were also allowed to react with the glucose oxidase reagent. A standard curve was created from the controls and the production of glucose from the hydrolysis of isomaltulose by the samples was estimated by comparing the absorbance at 500 nm for the samples to the standard curve.

Using this method, the alpha-1,6-glucosidase enzymes described by SEQ ID NOs: 1-6 were screened and found to have activities at temperatures ranging from 30 degrees C. to 80 degrees C. Table 4 describes the alpha-1,6-glucosidase activity measured in total cell lysate of an *E. coli* strain expressing the *Bacillus thermoamyloliquefaciens* enzyme (SEQ ID NO:5).

60 His Tagged Enzyme Recovery from Recombinant *E. coli*

Recombinant BL21 *E. coli* cells expressing an alpha-1,6-glucosidase (SEQ ID NOs: 1, 3, 5 and 6) were generated essentially as described in Example 1A. The frozen cell pellets expressing the his-tagged alpha-1,6-glucosidase key enzymes were brought up to a volume of 40 mL in extraction buffer (50 mM sodium phosphate, 500 mM NaCl, 10 mM Imidazole, pH 7.2-8 containing protease inhibitors (Roche

Complete EDTA-free protease inhibitor tablets)). Cells were lysed by 2 passages through a FRENCH Press (Thermo IEC). Cell lysates were centrifuged for 30 minutes at 10,000xg at 4 degrees C. Supernatants were collected and filtered using 0.45 micron vacuum filter device (Millipore).

A His Trap FF column was used to recover the his-tagged enzymes from the supernatant. A HisTrap FF 5 mL column (GE Healthcare) was equilibrated with extraction buffer. The clarified lysates were loaded at 5 mL/min. Bound his-tagged enzymes were eluted in 50 mM sodium phosphate, 500 mM NaCl, containing 150-500 mM Imidazole, pH 7.2-8.

The negative control was BL21[DE3] cell pellets transformed with empty pET24b vector essentially as described in Example 1A. Negative control cell pellets were extracted essentially as described above for the his-tagged alpha-1,6-glucosidase enzymes; however, the extraction buffer and elution buffers were at pH 7.2.

All samples collected from the HisTrap FF column were buffer exchanged into 50 mM HEPES, 50 mM NaCl, pH 7 using either Bio-Rad Econo-Pac 10-DG desalting column or HiPrep 26/10 desalting column (GE Healthcare). 50% Glycerol was added such that the final buffer was 40 mM HEPES, 40 mM NaCl, 10% glycerol, pH 7. Protein concentrations were estimated by Bradford assay. Samples were stored at -80 degrees C.

T. ethanolicus alpha-1,6-glucosidase (SEQ ID NO: 6):

His-tagged *T. ethanolicus* alpha-1,6-glucosidase (SEQ ID NO: 6) was recovered from recombinant BL21 *E. coli* cells essentially as described above (Example 2B "His tagged enzyme recovery from recombinant *E. coli*"). Frozen samples derived from the HisTrapFF column were combined with 3 M ammonium sulfate, 50 mM ammonium phosphate, pH 7 to a final ammonium sulfate concentration of 1 M. This sample was applied to a 5 mL HiTrap Phenyl HP column (GE Healthcare). Bound proteins were eluted from the column by washing the column with a linear ammonium sulfate gradient over 100 ml from 50 mM Sodium phosphate, 1.5 M ammonium sulfate, pH 7 to 50 mM sodium phosphate buffer pH 7 containing no ammonium sulfate. Fractions containing the enzyme were pooled and concentrated using Centri-prep YM-30 concentrator device (Amicon).

B. thurgiensis alpha-1,6-glucosidase (SEQ ID NO: 3):

His-tagged *B. thurgiensis* alpha-1,6-glucosidase (SEQ ID NO: 3) was recovered from recombinant BL21 *E. coli* cells essentially as described above (Example 2B "His tagged enzyme recovery from recombinant *E. coli*"). Fractions containing his-tagged enzyme were pooled and diluted in 50 mM HEPES, pH 6. Sample was applied to a 5 mL HiTrap Q HP column (GE Healthcare). Bound proteins were eluted by washing the column with a linear NaCl gradient over 100 mL from 50 mM HEPES, pH 6 to 50 mM HEPES, 1 M NaCl, pH 6. The fractions containing the enzyme were pooled.

G. thermoglucosidasius alpha-1,6-glucosidase (SEQ ID NO: 1):

His-tagged *G. thermoglucosidasius* alpha-1,6-glucosidase (SEQ ID NO: 1) was recovered from recombinant BL21 *E. coli* cells essentially as described above (Example 2B "His tagged enzyme recovery from recombinant *E. coli*"). Fractions containing his-tagged enzyme were pooled and diluted in 50 mM Tris-HCl, pH 7. Sample was applied to a 5 mL HiTrap Q HP column (GE Healthcare). Bound proteins were eluted by washing the column with a linear NaCl gradient over 100 mL from 50 mM HEPES, 10 mM NaCl, pH 7 to 50 mM HEPES, 1 M NaCl, pH 7. The fractions containing the enzyme were pooled and concentrated to 1 mL Centri-prep YM-30 concentrator device (Amicon). Sample was applied to a HiPrep 26/60 S-100 HR size exclusion column and eluted

with 20 mM Tris-HCl, 250 mM NaCl, pH 7. Fractions containing the enzyme were pooled and diluted in 1.5 M Ammonium Sulfate, 50 mM Sodium phosphate, pH7. Sample was applied to a 5 mL HiTrap Phenyl HP column (GE Healthcare). Bound proteins were eluted by washing the column with a linear ammonium sulfate gradient over 100 mL from 50 mM Sodium phosphate, 1.5 M ammonium sulfate, pH 7 to 50 mM sodium phosphate buffer pH 7 containing no ammonium sulfate. Fractions containing the enzyme were pooled.

B. thermoamyloliquefaciens alpha-1,6-glucosidase (SEQ ID NO: 5):

His-tagged *B. thermoamyloliquefaciens* alpha-1,6-glucosidase (SEQ ID NO: 5) was recovered from recombinant BL21 *E. coli* cells essentially as described above (Example 2B "His tagged enzyme recovery from recombinant *E. coli*"). Fractions containing his-tagged enzyme were pooled and diluted in 20 mM Tris-HCl, pH 7. Sample was applied to a 5 mL HiTrap Q HP column (GE Healthcare). Bound proteins were eluted by washing the column with a linear NaCl gradient over 100 mL from 20 mM Tris-HCl, 50 mM NaCl, pH 7 to 50 mM HEPES, 1 M NaCl, pH 7. Fractions containing the enzyme were pooled and concentrated to 1 mL Centri-prep YM-30 concentrator device (Amicon). Sample was applied to a HiPrep 26/60 S-100 HR size exclusion column and eluted with 50 mM HEPES, 50 mM NaCl, pH 7.4. Fractions containing the enzyme were pooled in 1.5 M Ammonium Sulfate, 50 mM Sodium phosphate, pH7. Sample was applied to a 5 mL HiTrap Phenyl HP column (GE Healthcare). Bound proteins were eluted by washing the column with a linear ammonium sulfate gradient over 100 mL from 50 mM Sodium phosphate, 1.5 M ammonium sulfate, pH 7 to 50 mM sodium phosphate buffer pH 7 containing no ammonium sulfate. Fractions containing the enzyme were pooled.

Activity of His-Tagged alpha-1,6-glucosidase Key Enzymes

The enzyme activity of the alpha-1,6-glucosidase enzymes (SEQ ID NOs: 1, 3, 5 and 6) recovered from recombinant BL21 *E. coli* cells was measured. Samples collected from the purification schemes described above (Example 2B) were diluted to 0.2 mg/mL in 50 mM HEPES, 50 mM NaCl, pH 7. Reactions were initiated by mixing samples with an equal volume of 100 mM HEPES, 4 mM EDTA, 0.04% Tween-20, 200 mM Isomaltulose, pH 7. For buffer controls, 100 mM HEPES, 4 mM EDTA, 0.04% Tween-20, pH 7 was combined with an equal volume of 200 mM Isomaltulose. Reactions were incubated at optimal temperature for the enzyme (37, 45, or 60 degrees C.) for 40 minutes in a Biorad Tetrad 2 thermocycler for the appropriate time. Reactions were terminated by heating samples at 95 degrees C. for 5 minutes. Glucose concentrations in reactions were estimated using the GOPOD assay. Enzyme activity is detected as the conversion of isomaltulose to glucose.

The GOPOD assay was performed by combining 20 uL aliquots of reaction samples, or glucose standards of known concentrations, with 250 uL GlucoseOx Reagent (Pointe Scientific) in a 96-well assay plate (Costar 3370) and incubated for 10 minutes at 37 degrees C. Absorbance at wavelength of 500 nm was measured using SpectraMax 384 Plus plate reader. Absorbance values of sample reactions were converted to glucose concentrations using the equation from a glucose standard curve generated by plotting the absorbance value versus the known glucose standard concentration. The activity of the various alpha-1,6-glucosidase enzymes is described in Table 5.

TABLE 5

Activity data for alpha-1,6-glucosidase enzymes		
Sample (SEQ ID NO)	Glucose (mM)	Reaction temperature in degrees C.
<i>T. ethanolicus</i> (6)	19.72	60
<i>G. thermoglucosidarius</i> (1)	29.16	60
Negative control	0.07	60
Buffer only negative control	0.03	60
<i>B. thurgiensis</i> (3)	23.35	37
Negative control	0.07	37
Buffer only negative control	0.01	37
<i>B. thermoamyloliquefaciens</i> (5)	1.17	45
Negative control	0.09	45
Buffer only negative control	0.01	45

Purification of His-Tagged alpha-1,5-glucosidase and alpha-1,1-glucosidase Key Enzymes.

Recombinant BL21[DE3] cell pellets expressing His-tagged alpha-1,5-glucosidase and alpha-1,1-glucosidase key enzymes were generated essentially as described in Example 1A. Frozen cell pellets were brought up to a volume of 30-40 mL in extraction buffer (50 mM sodium phosphate, 500 mM NaCl, 10 mM Imidazole, pH 7.2 containing protease inhibitors (Roche Complete EDTA-free protease inhibitor tablets)). Cells were lysed by 2 passages through a FRENCH Press (Thermo EC). Cell lysates were centrifuged for 30 minutes at 10,000× g at 4 degrees C. Supernatants were filtered using 0.45 micron vacuum filter devices (Millipore). A HisTrap FF 5 ml column (GE Healthcare) equilibrated with extraction buffer was used to clarify the lysates which were loaded at 5 mL/min. Bound his-tagged enzymes were eluted in 50 mM sodium phosphate, 500 mM NaCl, containing 300 mM Imidazole, pH 7.2. All samples were buffer exchanged into 50 mM HEPES, 50 mM NaCl, pH 7 using a HiPrep 26/10 desalting column (GE Healthcare). 50% Glycerol was added to such that the final buffer was 40 mM HEPES, 40 mM NaCl, 10% glycerol, pH 7. Protein concentrations were estimated by Bradford assay. Samples were stored at -80 degrees C.

As a negative control, BL21[DE3] cell pellets expressing the empty pET24b vector were processed as described above except for elution from HisTrap was in 50 mM sodium phosphate, 500 mM NaCl, containing 500 mM Imidazole, pH 7.2. Activity Analysis of His-tagged alpha-1,5-glucosidase and alpha-1,1-glucosidase Key Enzymes

Extracts of his-tagged enzymes were generated essentially as described above and were diluted to 0.08 mg/mL in 40 mM HEPES, 40 mM NaCl, 10% glycerol, pH 7. Enzyme activity assays were initiated by mixing samples with an equal volume of 100 mM HEPES, 4 mM EDTA, 0.04% Tween-20, 200 mM leucrose (for alpha-1,5-glucosidase key enzymes (SEQ ID NOs: 30-33)) or 135 mM trehalulose/67 mM isomaltulose mixture (for alpha-1,1-glucosidase key enzyme (SEQ ID NO: 34)), pH 7. Reactions were incubated at optimal temperature (70 degrees C. for alpha-1,5-glucosidase enzymes and 80 degrees C. for alpha-1,1-glucosidase key enzyme) for 40 minutes in a Biorad Tetrad 2 thermocycler for the appropriate time. Reactions were terminated by heating samples at 95 degrees C. for 5 minutes. Key enzyme activity was demonstrated by the conversion of a locked substrate (leucrose or trehalulose and/or isomaltulose) to glucose. Glucose concentrations in reactions were estimated using GOPOD assay essentially as described above. Table 6 outlines data which demonstrates that his-tagged alpha-1,5-glucosidase enzymes and alpha-1,1-glucosidase enzyme are active and convert locked sugar substrates to fermentable sugar.

TABLE 6

Conversion of locked sugars to glucose by his-tagged key enzymes.					
Sample name (SEQ ID NO:)	GK24 N-del (30)	GK24 (31)	HB27 (32)	HB8 (33)	Negative Control
Glucose Conc. (mM)	0.94	1.01	0.42	1.56	0.05
Sample name (SEQ ID NO:)	SAM1606 (34)	Negative control			
Glucose concentration (mM)	8.67	0.46			

2C: Dextranase (E.C. 3.2.1.11)

Dextranases are glycosidases which catalyze the exo or endohydrolysis of 1,6 alpha D glucosidic linkages in dextrans thus converting the dextran to smaller sugar molecules. A codon optimized polynucleotide sequence coding for a dextranase enzyme may be synthesized, cloned into an expression vector and expressed in *E. coli* essentially as described in Example 1A.

Dextranase enzyme activity assays will monitor the rate of isomaltose released from a dextran molecule during a hydrolysis reaction. HPLC size exclusion chromatography will also be employed to determine the level of dextran hydrolysis achieved by measuring the release of individual sugars.

Assays will be validated using a commercially available dextranase from *Penicillium* sp I.U.B.: 3.2.1.11 (Worthington Biochemical Corporation, N.J. 08701). The dextran hydrolysis can be measured by incubating 0.1 mL of 5-20 micrograms/mL of dextranase with 1.9 mL of commercially available dextran solution (substrate). Thermostability of dextranases will be tested in experiments performed at 60 to 70 degrees C. which are temperatures relevant to sugar mill sugarcane juice processing. Validated assays will be further optimized for detection of functional dextranases cloned and expressed in *E. coli*.

EXAMPLE 3

Transgenic Plants

3A: Transgenic Sugarcane

Embryogenic callus was produced from the immature leaf tissue of sugarcane. In greenhouse, cane was harvested by cutting off immature shoots at or above ground level and outer leaves and leaf sheaths were stripped. Basal nodes and emergent leaves were trimmed. In the laboratory (laminar flow cabinet), excess leaf sheaths were unfurled, nodes were trimmed and cane was sterilized (sprayed with 70% ethanol or immersed in 20% bleach for 20 minutes). Additional outer leaf sheaths were removed to expose inner 4-6 leaf rolls and leaf roll was cut to manageable size (12-15 mm in length). Remaining basal nodes and internodes were removed to expose the leaf roll region just above the apical meristem.

Transverse sections of the leaf roll were cut to form discs 0.5-1.0 mm in thickness, using not more than a 3.0 cm length of the leaf roll material. Leaf roll discs were plated onto MS media containing 2-3 mg/L of 2,4-D and cultured in the dark for 3-4 weeks. Leaf roll discs were cut or split apart at the time of initiation or 2 weeks following initiation and the resulting pieces spread across media to promote a more consistent and prolific embryogenic/proto-embryogenic culture response. After 3-4 weeks of culture, embryogenic callus was selectively excised from leaf disc rolls and sub-cultured on same (MS+2,4-D) media. Further selective subcultures were per-

formed every 2-3 weeks, dependent upon growth and development to produce additional cultures, until cultures reach 8-10 weeks of age.

Gene Delivery using the Biolistics PDS 2000 Particle Delivery Device for Sugarcane Transformation

Target embryogenic cultures were prepared for gene delivery by selecting high quality target tissue pieces and preculturing them for 3-6 days on fresh media before gene delivery.

At 2-5 hours prior to gene delivery, target tissues were arranged in a target pattern on high osmotic potential media containing MS basal salts and B5 Vitamins supplemented with sucrose 30 g/L and 0.2 M sorbitol and 0.2 M mannitol plus 2 mg/l 2,4-D.

To prepare DNA for bombardment, gold particles (0.6 micrometer size, Bio-Rad) were re-suspended in 50% sterile glycerol by vortexing. An aliquot of the glycerol—gold particle suspension was combined by gentle mixing with 2×10^{10} mol DNA of the gene encoding the selectable marker (PMI) and genes of interest outlined in Table 29 of Example 12. The mixture was combined with 2.5M CaCl₂ and cold 1M spermidine to precipitate the DNA onto the gold particles. The gold particles with precipitated DNA were washed with ethanol. The gold particles were repeatedly re-suspended in ethanol and aliquots of DNA/particle suspension were placed evenly onto the center of individual macrocarrier membrane disks and allowed to dry. The macrocarrier was loaded into the gene gun above the stopping screen. Bombardment of embryos was performed with a PDS—1000 Helium gene gun. A rupture disc of 1300 psi was used and the distance from the rupture disc and the macrocarrier was set at 8 mm with a stopping screen at 10 mm. The distance between the stopping screen and the embryos was about 7 cm. The pressure on the helium tank was set at about 1400 psi. Target tissues (embryogenic cultures) were bombarded with 2 shots before being transferred to the dark at 28 degrees C. for about 12 hours.

After recovery, the bombarded cultures were transferred to maintenance medium and cultured at 28 degrees C. in the dark. After 7 days, the bombarded cultures were transferred to fresh selection medium containing mannose (7-9 grams/L), 5 g/L sucrose plus 2 mg/L 2,4-D and incubated for 4-5 weeks in dark. Growing callus pieces were then subcultured to fresh selection media every 2 weeks until they were large enough for analysis. Typically, 2 to 3 rounds of subculture were required.

Regeneration of Plants from Transgenic Callus Lines

After 4-5 weeks on mannose selection media, surviving embryogenic callus colonies are selectively isolated from original cultures and transferred onto regeneration media (MS salts and B5 vitamins, 30 g/L sucrose, supplemented with 3-6 g/L mannose and 2 mg/L BAP) at 28 degrees C. in dark in Flambeau boxes.

One week later, the cultures are transferred to a light room for shoot development under 16 hours light at 28 degrees C. After 3-4 weeks in the regeneration media, the visible green buds or shoots are sub-cultured on elongation media (MS basal salts and B5 vitamins, sucrose 30 g/L with hormone-free).

Regenerated shoots are rooted in the rooting media (Basal MS media). The rooting cultures are kept at 28 degrees C. under light for another 2 weeks before transfer to the greenhouse and soil. Any of the genes described in Example 1, Example 2 or Example 12 can be transformed into sugarcane to generate transgenic plants using the above described protocol. *Agrobacterium* mediated genetic transformation is also possible and methods are described in the literature such as Arencibia, Ariel D. and Carmona, Elva R. Sugarcane (*Sac-*

charum spp.) Methods in Molecular Biology (Totowa, N.J., United States) (2006), 344(*Agrobacterium* Protocols (2nd Edition), Volume 2), 227-235

3B: Transgenic Sugarcane Expressing Dextranase Activity

Selected dextranases are sequence optimized based upon the codon preference for sugarcane. The sugarcane codon optimized sequence is cloned into transformation vectors for sugarcane transformation. One of skill in the art is able to select the appropriate promoter and terminator for the dextranase gene as well as select an appropriate selectable marker for sugarcane transformation. Targeting sequences are incorporated into the expression construct for dextranases to target the enzyme to the vacuolar compartment of parenchyma cells where sucrose is stored.

Transgenic sugarcane plants are generated as described in Example 3A. Transformed plants are analyzed using routine methods for DNA analysis of transgenic plants in order to determine if the expression construct has been incorporated into the nuclear DNA of the sugarcane plant.

Transgenic sugarcane plants are evaluated for dextranase enzyme activity. Mature plant tissue is crushed and the juice will be collected and chilled prior to assaying for dextran accumulation using the detection methods described in Example 1C. Enzyme assay methods described in Example 1C are used to determine the functionality of the expressed enzyme in transgenic plants.

3C: Generation of Transgenic Plants Expressing Dextranase Activity.

Selected dextranases are codon optimized for expression in sugarcane using the codon preference for sugarcane. The sugarcane optimized gene sequence is cloned into a transformation vector designed for sugarcane transformation. One of skill in the art is able to select the appropriate promoter and terminator for the dextranase as well as select an appropriate selectable marker for sugarcane transformation. The dextranase enzyme is targeted to the ER subcellular compartment of parenchyma cells using the appropriate targeting sequences. The dextranase enzyme is targeted away from the sucrose and dextran storage compartment of the sugarcane plant.

Transgenic plants are generated as described in Example 3A. Enzyme activity is evaluated in mature plant tissue by crushing and extracting juice from the transgenic plant and performing the assays for dextranase activity as described in Example 2C. Enzyme assay methods described in Example 2C are used to determine the functionality of the expressed enzyme in sugarcane juice 3D: Transient expression in tobacco and sugar beet leaves

Expression cassettes described in Example 12 were cloned into either a binary vector or a binary vector also containing an origin of replication from BCTV, beet curly top virus, (SEQ ID NO: 8). The binary vectors without the origin of replication from BCTV were transferred into *Agrobacterium tumefaciens* strain LBA4404 using the freeze-thaw method (An et al., Binary vector. In: Gelvin S B, Schilproot R A (eds), Plant molecular biology manual. Kluwer Academic Publishers, Dordrecht, pp A3 1-19 (1988)). The binary vectors containing the origin of replication from BCTV (BCTV binary vectors) were transferred into *Agrobacterium tumefaciens* strain LBA4404 containing a helper plasmid containing a replicase sequence from BCTV (SEQ ID NO: 9) using the freeze-thaw method (An et al., Binary vector. In: Gelvin S B, Schilproot. RA (eds), Plant molecular biology manual. Kluwer Academic Publishers, Dordrecht, pp A3 1-19 (1988)).

Leaves from sugar beet or tobacco were used for the transient expression of enzymes in plant tissue. Tobacco leaves from transgenic TEV-B tobacco plants (made in the tobacco

cultivar Xanthi) containing a mutated PI/HC-Pro gene from TEV that suppresses post-transcriptional gene silencing (Mallory et al., Nat Biotechnol 20:622 (2002)) were used for transient expression of selected enzymes. Preparation of *Agrobacterium* cultures and infiltration of tobacco or sugar beet leaves was carried out as described by Azhakanandam et al., Plant Mol. Biol. 63: 393-404 (2007). In brief, the genetically modified *agrobacteria* were grown overnight in 50 mL of LB medium containing 100 μ M acetosyringone and 10 μ M MES (pH 5.6), and subsequently were pelleted by centrifugation at 4000 \times g for 10 min. The pellets were resuspended in the infection medium [Murashige and Skoog salts with vitamins, 2% sucrose, 500 μ M MES (pH 5.6), 10 μ M MgSO₄, and 100 μ M acetosyringone] to OD₆₀₀=1.0 and subsequently held at 28 degrees C. for 3 hours. Infiltration of individual leaves was carried out on sugar beet (about 3 weeks old) and TEV-B tobacco plants (about 4 weeks old) using a 5 mL syringe by pressing the tip of the syringe (without a needle) against the abaxial surface of the leaf. Infiltrated plants were maintained at 22-25 degrees C. with a photoperiod of 16 hours light and 8 hours dark. Plant tissue was harvested after 5 days post infiltration for subsequent analysis.

To ensure that enzyme activity measured was due to plant expression of the enzymes, the expression constructs also incorporated an intron in the polynucleotide sequence coding for the enzyme. The presence of the intron ensures that expression of the enzyme is due to plant expression (able to process out the intron and therefore express a fully processed enzyme) versus *agrobacterium* expression (unable to process the intron and thus not able to express a functional enzyme). 3D: Transient Expression in Tobacco and Sugar Beet Leaves

Expression cassettes described in Example 12 were cloned into either a binary vector or a binary vector also containing an origin of replication from BCTV, beet curly top virus (SEQ ID NO: 8). The binary vectors without the BCTV origin of replication were transferred into *Agrobacterium tumefaciens* strain LBA4404 using the freeze-thaw method (An et al., Binary vector. In: Gelvin S B, Schilpoot R A (eds), Plant molecular biology manual. Kluwar Academic Publishers, Dordrecht, pp A3 1-19 (1988)). The BCTV containing binary vectors were transferred into *Agrobacterium tumefaciens* strain LBA4404 containing a helper plasmid containing a BCTV replicase sequence (SEQ ID NO: 9) using the freeze-thaw method (An et al., Binary vector. In: Gelvin S B, Schilpoot R A (eds), Plant molecular biology manual. Kluwar Academic Publishers, Dordrecht, pp A3 1-19 (1988)).

Leaves from sugar beet or tobacco were used for transient expression of enzymes. Transgenic TEV-B tobacco plants (made in the tobacco cultivar Xanthi) containing a mutated PI/HC-Pro gene from TEV that suppresses post-transcriptional gene silencing (Mallory et al., Nat Biotechnol 20:622 (2002)) were used for transient expression of selected enzymes in tobacco leaves. Preparation of *Agrobacterium* cultures and infiltration of tobacco or sugar beet plants was carried out as described by Azhakanandam et al., Plant Mol. Biol. 63: 393-404 (2007). In brief, the genetically modified *agrobacteria* were grown overnight in 50 mL of LB medium containing 100 μ M acetosyringone and 10 μ M MES (pH 5.6), and subsequently were pelleted by centrifugation at 4000 \times g for 10 min. The pellets were resuspended in the infection medium [Murashige and Skoog salts with vitamins, 2% sucrose, 500 μ M MES (pH 5.6), 10 μ M MgSO₄, and 100 μ M acetosyringone] to OD₆₀₀=1.0 and subsequently held at 28 degrees C. for 3 hours. Infiltration of individual leaves was carried out on sugar beet (about 3 weeks old) and TEV-B tobacco plants (about 4 weeks old) using a 5 mL syringe by pressing the tip of the syringe (without a needle) against the

abaxial surface of the leaf. Infiltrated plants were maintained at 22-25 degrees C. with a photoperiod of 16 hours light and 8 hours dark. Plant tissue was harvested after 5 days post infiltration for subsequent analysis.

5 3E. Maize Transient Expression System

Expression cassettes described in Example 12 were cloned into a binary vector. The constructs were transferred into *Agrobacterium tumefaciens* strain LBA4404 containing helper plasmid (pSBI) using a freeze-thaw method (An et al., Binary vector. In: Gelvin S B, Schilpoot R A (eds), Plant molecular biology manual. Kluwar Academic Publishers, Dordrecht, pp A3 1-19 (1988)).

The maize transient expression system was established using young maize seedlings (5-12 d old). Preparation of *Agrobacterium* cultures and infiltration of maize leaves was carried out as described by Azhakanandam et al., Plant Mol. Biol. 63: 393-404 (2007). In brief, the genetically modified *agrobacteria* were grown overnight in 50 mL of LB medium containing 100 μ M acetosyringone and 10 μ M MES (pH 5.6), and subsequently were pelleted by centrifugation at 4000 \times g for 10 min. The pellets were resuspended in the infection medium (Murashige and Skoog salts with vitamins, 2% sucrose, 500 μ M MES (pH 5.6), 10 μ M MgSO₄, and 100 μ M acetosyringone) to OD₆₀₀=1.0 and subsequently held at 28 degrees C. for 3 hours. Infiltration of individual leaves was carried out on maize seedlings using a 5 mL syringe, without a needle, by pressing the tip of the syringe against the abaxial surface of the leaf. Infiltrated plants were maintained at 22-25 degrees C. with a photoperiod of 16 hours light and 8 hours dark. Plant tissue was harvested after 5-7 days post infiltration for subsequent analysis.

To ensure that enzyme activity measured was due to plant expression of the enzymes, the expression constructs also incorporated an intron in the polynucleotide sequence coding for the enzyme. The presence of the intron ensures that expression of the enzyme is due to plant expression (able to process out the intron and therefore express a fully processed enzyme) versus *agrobacterium* expression (unable to process the intron and thus not able to express a functional enzyme). 3F. Transgenic Maize Callus and Plants

Transformation of maize callus was performed using a biolistic transformation method. Maize embryos were collected from maize kernels about 8 to 11 days after pollination. The ears were collected and sterilized in 20% Germicidal Clorox for 20 minutes on an orbital shaker set at 120 rpm followed by extensive rinsing of the ear in sterile water. Embryos were collected from the kernels and kept on culture media in the dark for 3 to 7 days.

To prepare DNA for bombardment, gold particles (0.6 to 1 micrometer size, Bio-Rad) were resuspended in 50% sterile glycerol by vortexing. An aliquot of the glycerol—gold particle suspension was combined by gentle mixing with 2 \times 10¹⁰ mol DNA of the gene encoding the selectable marker (PM) and gene of interests outlined in Table 29 of Example 12. The mixture was combined with 2.5M CaCl₂ and cold 1M spermidine to precipitate the DNA onto the gold particles. The gold particles with precipitated DNA were washed in ethanol. The washed gold particles were re-suspended in ethanol and aliquots of DNA suspension were placed evenly onto the center of individual macrocarrier membrane disks and allowed to dry. The macrocarrier was loaded into the gene gun above the stopping screen. Bombardment of embryos was performed with a PDS Helium—1000 gene gun. A rupture disc in the range of 650-1800 psi was used and the distance from the rupture disc and the macrocarrier was set at 8 mm with a stopping screen at 10 mm. The distance between the stopping screen and the embryos was about 7 cm. The pres-

sure on the helium tank was set at about 1200 psi. Target tissues (embryos) were bombarded 3 times before being transferred to the dark at 28 degrees C. to recover for 3 days.

After recovery, the bombarded embryos were transferred to maintenance medium and cultured at 28 degrees C. in the dark. After 3 days, the bombarded embryo tissue was transferred to fresh callus induction medium and incubated for 1 week to induce callus formation. The calli were then transferred to selection medium containing mannose for three weeks at 28 degrees C. in the dark.

Selection of transgenic calli was performed by transferring living callus tissue to selection medium and cultured at 28 degrees C. in the dark for 3 weeks. Surviving calli were transferred to fresh selection medium and cultured an additional 2 weeks at 28 degrees C. in the dark. Surviving calli were then transferred to regeneration medium and cultured at 28 degrees C. in the dark for 2 weeks.

Callus tissues will be incubated under 16 hours of light at 24 degrees C. to encourage shoot development. Once shoot development starts, callus with shoots will be transferred to rooting medium and cultured at 24 degrees C. with light for another week prior to transplanting to soil for the remainder of the maize growing cycle.

3G: Analysis of Key Enzymes in Plant Tissue

Whole leaves from tobacco or sugar beet transiently expressing an enzyme were frozen at -80 degrees C. in 24-well blocks containing 3/16" chrome ball bearings. The frozen material was shaken at setting 9 for 2 min in a Kleco Titer plate/Microtube Grinding Mill creating a powder. Buffer (50 mM HEPES, 2 mM EDTA, 0.02% Tween-20, 100 mM locked sugar (isomaltulose, leucrose, or trehalulose depending upon the enzyme), pH 7) was added to the powdered samples to give a thick slurry. Samples were incubated in a Glas-Col rotator at 80% speed for 30 min. Samples were transferred by wide-bore P200 pipet to PCR tubes at 100 uL per tube and incubated at the appropriate temperature for the enzyme (50, 60, 70, 80 degrees C. depending on enzyme) in a Biorad Tetrad 2 thermocycler. The sample was transferred to either a Millipore Biomax 5KD MW membrane spin filter and centrifuged at 12,000xg for 20 min or a Millipore Multiscreen-HV filter plate and filtered at 20 InHg vacuum. After filtration, the samples were diluted in Milli-Q water as necessary and placed into either 0.3 or 1.5 mL sample vials with split caps for carbohydrate analysis by Dionex HPAEC.

3H: Analysis of Locking Enzymes in Plant Tissue

Whole leaves from tobacco, sugar beet, or maize were rolled and placed into filtration baskets (DNA IQ Spin Basket) and the filled filtration baskets placed into 1.5 mL eppendorf tubes. The filled filtration baskets and eppendorf tubes were frozen on dry ice for 5-8 min (or until frozen) followed by thawing on ice for 5-8 min (or until thawed). The thawed filled filtration baskets and eppendorf tubes were then centrifuged at 10,000xg for 15 min at 4 degrees C. and the filtrate collected.

The filtrate was boiled at 100 degrees C. for 5 min followed by centrifugation at 16,000xg for 20 min. The boiled filtrate was further filtered by transferring the boiled filtrate to either a Millipore Biomax 5 KD MW membrane spin filter and centrifuged at 12,000xg for 20 min or a Millipore Multiscreen-HV filter plate and filtered at 20 InHg. The filtrate was collected and diluted in Milli-Q water as necessary and placed into either 0.3 or 1.5 mL sample vials with split caps for analysis.

EXAMPLE 4

Plant Expressed Sucrose Isomerase Enzyme

4A: Transient Expression of Sucrose Isomerase in Sugar Beet and Tobacco Leaves

The transformation vector 17588, as described in Example 12, was used to transiently expressing enzymes in tobacco or sugar beet leaves essentially as described in Example 3D. Tobacco or sugar beet leaves transiently expressing a sucrose isomerase were generated using the vector 17588 which contains a dicot optimized polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 16). Leaves transiently expressing sucrose isomerase were harvested and extracted essentially as described in Example 3H and analyzed by Dionex for carbohydrates essentially as described in Example 1G.

Dionex HPAEC chromatography utilized pure sugar standards as a reference for retention time and standard curve production for determining sugar concentrations. Sugar concentrations were based on the total sugar consisting of glucose, fructose, sucrose, trehalulose and isomaltulose when present. These five sugars represent >98% of the total peak area of the chromatograms with the remainder coming from minor unknown peaks from the biological extraction milieu of the leaf.

Sucrose isomerase activity in transiently infiltrated leaves was directly detected by the formation of the two major products of the enzymatic conversion of sucrose to the locked sugars, trehalulose and isomaltulose. Neither of the locked sugars were present in control leaves. Tables 7-10 summarize the analysis of tobacco and sugar beet transiently expressing a sucrose isomerase (vector 17588) and demonstrate that tobacco and sugar beet plants are able to express an active sucrose isomerase which catalyzes the conversion of sucrose to the locked sugars isomaltulose and trehalulose and accumulate the locked sugars in the leaves.

TABLE 7

Carbohydrate analysis (HPAEC) of tobacco leaves expressing a sucrose isomerase (SEQ ID NO: 16).				
sample	Sucrose (mM)	Trehalulose (mM)	Isomaltulose (mM)	Total Disaccharide (mM)
17588	3.6	17.7	6.4	27.7
17588	6.8	34.3	14.1	55.2
17588	4.2	23.9	8.1	36.2
17588	14.7	33.1	13.8	61.6
Negative control	11.9	0.0	0.0	11.9
Negative control	11.8	0.0	0.0	11.8
Negative control	6.3	0.0	0.0	6.3
Negative control	4.2	0.0	0.0	4.2

TABLE 8

Carbohydrate analysis (HPAEC) of tobacco leaves transiently expressing sucrose isomerase.				
sample	Glucose + Fructose (% total sugar)	Sucrose (% total sugar)	Trehalulose (% total sugar)	Isomaltulose (% total sugar)
17588	39.2	7.9	38.8	14.1
17588	51.4	6.0	30.2	12.4

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TABLE 8-continued

Carbohydrate analysis (HPAEC) of tobacco leaves transiently expressing sucrose isomerase.				
sample	Glucose + Fructose (% total sugar)	Sucrose (% total sugar)	Trehalulose (% total sugar)	Isomaltulose (% total sugar)
17588	47.9	6.0	34.4	11.7
17588	51.7	11.5	26.0	10.8
Negative control	40.6	59.4	0.0	0.0
Negative control	58.5	41.5	0.0	0.0
Negative control	45.7	54.3	0.0	0.0
Negative control	53.3	46.7	0.0	0.0

TABLE 9

Carbohydrate analysis (HPAEC) of sugar beet leaves transiently expressing sucrose isomerase (SEQ ID NO: 16).				
Sample	Sucrose (mM)	Trehalulose (mM)	Isomaltulose (mM)	Total disaccharide (mM)
17588	8.5	9.9	3.1	21.5
17588	16.6	0.7	0.1	17.3
17588	15.1	2.5	1.3	18.9
17588	31.8	0.5	0.3	32.6
Negative control	10.0	0.0	0.0	10.0
Negative control	15.3	0.0	0.0	15.3
Negative control	17.6	0.0	0.0	17.6
Negative control	7.8	0.0	0.0	7.8

TABLE 10

Carbohydrate analysis (HPAEC) of sugar beet leaves transiently expressing sucrose isomerase (SEQ ID NO: 16).				
Sample	Glucose + fructose (% total sugar)	Sucrose (% total sugar)	Trehalulose (% total sugar)	Isomaltulose (% total sugar)
17588	28.2	28.5	33.1	10.2
17588	43.2	54.2	2.3	0.3
17588	56.5	34.7	5.8	3.0
17588	42.4	56.1	0.9	0.6
Negative control	50.4	49.6	0.0	0.0
Negative control	42.9	57.1	0.0	0.0
Negative control	39.8	60.2	0.0	0.0
Negative control	74.4	25.6	0.0	0.0

4B: Transient Expression of Enzymes in Maize Leaves

Transient expression of enzymes in maize leaves was performed essentially as described in Example 3E using the binary vector pEB47 (described in Example 12) comprising a monocot optimized polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 24). Maize leaves were harvested and analyzed for the presence of isomaltulose and trehalulose (products of sucrose isomerase activity within the maize leaf) essentially as described above for tobacco and sugar beet leaves transiently expressing sucrose isomerase. Table 11 outlines data that demonstrates sucrose isomerase is actively expressed in maize leaves transiently expressing

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sucrose isomerase and leads to the accumulation of the locked sugars, isomaltulose and trehalulose within the maize leaf.

TABLE 11

Carbohydrate analysis (HPAEC) of maize leaves transiently expressing sucrose isomerase (SEQ ID NO: 24).				
Sample	Glucose + fructose (% total sugar)	Sucrose (% total sugar)	Trehalulose (% total sugar)	Isomaltulose (% total sugar)
47-6 (pEB47)	78.9	17.2	2.4	1.5
47-7 (pEB47)	63.7	33.3	2.1	0.9
47-8 (pEB47)	73.1	16.0	7.3	3.6
Negative control (GUS containing construct)	69.4	30.6	0.0	0.0
Negative control leaf tissue	58.2	41.8	0.0	0.0

4C: Transgenic Maize Callus Expressing Sucrose Isomerase

Transgenic maize callus expressing sucrose isomerase was generated by bombarding maize embryos with linear polynucleotide sequence. The method of embryo transformation and generation of callus was essentially as described in Example 3F; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic maize cells by growth on mannose. The second polynucleotide sequence, pEB38, contained a maize optimized polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 20). The sucrose isomerase was targeted to the vacuole. Table 12 outlines data which demonstrates that transgenic maize callus which expresses sucrose isomerase accumulated the locked sugars trehalulose and isomaltulose.

TABLE 12

Carbohydrate analysis (HPAEC) of transgenic maize callus tissue expressing sucrose isomerase.				
Sample	Glucose + Fructose % total sugar	Sucrose % total sugar	Trehalulose % total sugar	Isomaltulose % total sugar
1 pEB38	14.8	0.95	38.2	46.0
2 pEB38	25.0	0.69	35.3	39.0
3 pEB38	32.0	5.13	34.8	28.1
Negative control	70.0	30.0	0.0	0.0

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control is transgenic maize callus generated by bombardment with the polynucleotide sequence encoding PMI only.

4D: Transgenic Sugarcane Callus Expressing Sucrose Isomerase

Transgenic sugarcane callus expressing sucrose isomerase was generated essentially as described in Example 3A; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic sugarcane cells by growth on mannose. The second polynucleotide sequence, pEB38, contained a monocot optimized polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 20). The sucrose isomerase was targeted to the vacuole. Table 13 outlines data which demonstrates that transgenic sugarcane callus which expresses sucrose isomerase accumulated the locked sugars trehalulose and isomaltulose.

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TABLE 13

Carbohydrate analysis (HPAEC) of transgenic sugarcane callus tissue expressing sucrose isomerase.				
Sample	Glucose + Fructose % total sugar	Sucrose % total sugar	Trehalulose % total sugar	Isomaltulose % total sugar
1 pEB38	44.13	37.70	8.87	9.30
Negative control	34.61	65.39	0.0	0.0

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control is transgenic sugarcane callus generated by bombardment with a polynucleotide sequence encoding the selectable marker PMI.

4E: Transgenic Sugar Beet Expressing Sucrose Isomerase (SEQ ID NO: 16)

Transgenic sugar beet plants containing the expression cassette 17588 (described in Example 12) were generated essentially as described in patent application WO02/14523 which is a multiple shoot method of transformation. The transgenic sugar beet callus was selected using mannose selection (the selectable marker gene was PMI) which was performed essentially as described in patent application WO94/20627.

The transgenic sugar beet plants were analyzed by PCR to determine if the selectable marker (PMI) and the sucrose isomerase gene (SEQ ID NO: 16) were present in the plant. In addition, the transgenic sugar beet plants were analyzed for the accumulation of locked sugars.

To analyze the sugar content of the transgenic sugar beet plants, leaves from the transgenic sugar beet plants were sampled into a Costar 96-well box. The box was placed on ice during the sampling procedure. After filling the box with glass balls the leaf samples were placed into the wells and 100 μ L sterile ddH₂O was added. The wells were closed using strip caps or a lock and the box shaken in a Tissue laser (25-30 s, 30 Hz.) to pulverize the tissue in the water. The locks covering the wells were pierced and the samples were boiled on a water bath for 10 min. After boiling, an additional 100 μ L sterile ddH₂O was added followed by centrifugation (10 min, 3000 rpm). The supernatants were transferred to Millipore spin filter and centrifuged at 12000 rpm, 5 min. The filtered supernatants were stored at -20 degrees C. or in 4 degrees C. if the analysis was performed directly.

The samples were diluted 100 times with distilled water prior to analysis using the Dionex HPAE-system. The Dionex HPAE-system, ICS-3000 was used to separate the carbohydrates. The instrument was equipped with a temperature regulated auto sampler, CarboPac PA20 3 \times 30 mm guard column, CarboPac PA20 3 \times 15 mm analytical column and pulsed amperometric detector (PAD). The mobile phase used was 200 mM NaOH solution and water in following gradient program: 8 min/16% NaOHsolution/2 min 16-100% NaOHsolution/3 min 100% NaOHsolution/2 min 100-16%/7 min 16% NaOHsolution. The column temperature was set at 30 degrees C. and the flow 0.43 mL/min. The approximate retention times were glucose 7.7 min, fructose 9.3 min, sucrose 11.0 min, trehalulose 13.1 min and isomaltulose 14.5 min. The peaks were identified using the standard solutions. Table 14 outlines data which demonstrates transgenic sugar beet plants expressing a sucrose isomerase enzyme and the subsequent accumulation of the locked sugars, isomaltulose and trehalulose. Locked sugars are detected in transgenic sugar beet plants expressing sucrose isomerase indicating that the enzyme is both expressed and is able to perform the enzymatic activity which converts sucrose to isomaltulose and trehalulose.

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TABLE 14

Transgenic sugar beet plants expressing sucrose isomerase.				
Event	PCR PMI	PCR GOI	Dionex-isomaltulose	Dionex-trehalulose
0851B:1 A biennial	+	+	+	++
0851B:2 A biennial		+	+	++
0851F:2 A biennial	+	+	-	+
0851I:1 B biennial	+	-	+	++
0851K:2 A biennial	+	+	++	+++
0851K:2 B biennial	+	-	-	-
0851K:2 C biennial	+	-	-	-
0851K:4 A biennial	+	+	-	+
0851N:1 A biennial		+	-	+
0851O:1 A biennial	+	+	+	++
0851O:2 A biennial	+	+	+	++
0851O:3 A biennial	+	+	+	+++
0851O:4 A biennial		+	+	++
0851O:5 A biennial	+	+	+	++
0903B:5 A annual		-	+	++
0903B:7 A annual		+	+	+
0903D:1 A annual	+	+	-	+
0903F:1 B annual	+	+	+	++
0903F:1 C annual	+	+	+	++
0903G:1 A annual	+	+	-	+
0903I:1 A annual	+	+	+	++

EXAMPLE 5

Transgenic Plants Expressing Dextranucrase with Leucrose Synthase Activity

5A: Transient Expression of Dextranucrase (SEQ ID NO: 35) in Tobacco Leaves

The transformation vector 902195, as described in Example 12, was used to generate tobacco leaves transiently expressing dextranucrase essentially as described in Example 3D. Transient expression of dextranucrase in tobacco leaves was performed using the vector 902195 which contains a dicot optimized polynucleotide sequence encoding a dextranucrase with leucrose synthase activity (SEQ ID NO: 35). Transiently expressing leaves were harvested and extracted essentially as described in Example 3H and analyzed by Dionex for carbohydrates essentially as described in Example 1G.

Dionex HPAE chromatography utilized pure sugar standards as a reference for retention time and standard curve production for determining sugar concentrations. Sugar concentrations were based on the total sugar consisting of glucose, fructose, sucrose, and locked sugars when present. These sugars represent >98% of the total peak area of the

chromatograms with the remainder coining from minor unknown peaks from the biological extraction milieu of the leaf.

Dextranase with leucrose synthase activity transiently expressed in leaves was directly detected by the formation of the locked sugar leucrose. Leucrose was not present in control leaves. Table 15 summarizes the analysis of tobacco leaves transiently expressing a dextranase with leucrose synthase activity (vector 902195) and demonstrates that tobacco leaves are able to express an active dextranase which catalyzes the conversion of sucrose to the locked sugar leucrose which accumulates in the leaf.

5B: Transient Expression of Dextranase (SEQ ID NO: 24) in Maize Leaves.

Maize leaves transiently expressing dextranase with leucrose synthase activity were generated essentially as described in Example 3E using the vector pEB47 (described in Example 12) comprising a monocot optimized polynucleotide sequence encoding a dextranase (SEQ ID NO: 47). Maize leaves were harvested and extracted essentially as described in Example 3H. The extract was analyzed for carbohydrate content essentially as described in Example 1G. Table 15 outlines data that demonstrates dextranase is actively expressed in maize leaves and leads to the accumulation of the locked sugar leucrose within the maize leaf.

5C: Transgenic Sugarcane Callus Expressing Dextranase (SEQ ID NO: 37)

Transgenic sugarcane callus expressing dextranase with leucrose synthase activity (SEQ ID NO: 37) was generated essentially as described in Example 3A; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic sugarcane cells by growth on mannose. The second polynucleotide sequence, pEB28, contained a monocot optimized polynucleotide sequence encoding a dextranase (SEQ ID NO: 37). The dextranase was targeted to the vacuole. Table 15 outlines data which demonstrates that transgenic sugarcane callus which expresses sucrose isomerase accumulated the locked sugar leucrose.

TABLE 15

Plant tissue expressing dextranase accumulates leucrose and/or isomaltose.			
	tobacco	maize	sugar cane
dextranase	Leucrose	Leucrose	Leucrose and isomaltose
Negative control	—	—	—

Leucrose synthase activity is determined by the accumulation of leucrose above 10x signal: noise on a Dionex IC.

EXAMPLE 6

Transgenic Plants Expressing Amylosucrase

6A: Total Starch Analysis of Amylosucrase-Expressing Maize and Sugarcane Callus

The effectiveness of the amylosucrase gene, when expressed in either maize or sugar cane callus, can be evaluated by comparing the total starch content of the amylosucrase expressing calli to control calli that have not been transformed with the gene. The total starch content of any plant tissue of interest can be measured using a protocol similar to that of the Megazyme Total Starch Assay kit. In this assay, the starch contained in a plant sample is broken down into glu-

cose monomers through digestion by both an alpha-amylase and an amyloglucosidase. The resulting solution of glucose can be enumerated by a glucose oxidase-peroxidase (GO-POD) reaction essentially as is described in Example 2B. In this reaction, the glucose oxidase enzymes break down glucose to hydrogen peroxide which the peroxidase then digests, releasing oxygen which reacts with the 4-aminoantipyrine in solution to evolve a pink color. The pink color can be measured with a spectrophotometer and, when compared with the absorbance of a glucose standard, can give a measure of the amount of glucose and therefore, the amount of starch in a given sample.

To accurately measure the production of carbohydrate polymers by the amylosucrase gene in callus, several controls and conditions will need to be established. For every calli that is transformed with the amylosucrase gene, a duplicate calli should be transformed with an empty vector that can act as a control sample. Both transformed and control calli should initially be grown on sucrose media to provide amylosucrase with its natural substrate and raise the overall starch content in the calli. After sufficient growth, some calli (both AMS and control) should be transferred to sorbitol media where the natural metabolism of the tissue will lower the background of transient starch and, theoretically, leave the amylosucrase produced carbohydrate polymer. In tissue culture, sorbitol is assimilated and metabolized by plants to a much lesser degree than sucrose. With sorbitol as a carbon source, plant cells are expected to deplete transient and storage starch reserves leaving an amylosucrase derived starch to accumulate.

Once the calli are harvested from the media, similar events can be pooled into wells of a 24-well block to bulk up the amount of tissue and lyophilized so that calculations can be made on a dry weight basis. Lyophilized tissue can be easily ground in the 24-well blocks using a Kleco. As mentioned previously, the Megazyme total starch protocol can be used to effectively measure the total starch content of tissue samples. The following is an example of a slightly modified protocol that could be employed to analyze lyophilized callus material. Approximately 30-70 mg of the ground tissue should be washed with 5 mL of 80-90% ethanol for 30-60 minutes and centrifuged for 5 minutes at 3000 rpm to wash away any soluble sugars or other soluble compounds. Additional ethanol washes may be added as necessary, as long as all samples are treated identically. The pelleted material should then be washed in 5 mL of cold water and centrifuged again for 5 minutes at 3000 rpm to remove any remaining ethanol. At this stage, the pellet should be completely resuspended in 3 mL of a 1:30 dilution of alpha-amylase (Megazyme) in 50 mM MOPS buffer pH=7 and incubated for 6 minutes in a 100 degree C. water bath. Samples should then be transferred to a 50 degree C. water bath where 4 mL of NaOAc buffer pH=4.5 and 0.1 mL of amyloglucosidase (Megazyme) will be added and then incubated for 30 minutes at 50 degree C. After incubation, all samples should be brought to 10 mL with water, vortexed, and centrifuged for 10 minutes at 3000 rpm. This supernatant contains the solubilized glucose monomers that remain from the digestion of the carbohydrate polymers that were extracted from the lyophilized tissue samples. To enumerate the glucose in this mixture, 2 mL should be added in duplicate to glass test tubes, mixed with 3 mL of GOPOD reagent, and incubated for 20 minutes at 50 degree C. Once cooled to room temperature, the optical density of the samples can be read at 510 nm. Based on the OD reading of the samples and its comparison to a known standard, the amount of glucose, and therefore starch, in the original dry weight sample can be calculated.

Upon completion of total starch content analysis, it is expected that calli expressing the amylosucrase gene will show an increased level of total starch over the negative control calli due to the additional production of carbohydrate polymers by the enzyme. Additionally, targeted expression of the amylosucrase enzyme to the vacuole or apoplast of transgenic plant cells would serve to isolate the de novo starch from the endogenous starch metabolizing enzymes allowing for accumulation of a locked carbohydrate. Therefore, when the calli are depleted of transient starch after growth on sorbitol media, the total starch content would be expected to fall slightly, but remain at an increased level over the negative controls.

6B: Starch Structure: Amylose/Amylopectin Differentiation by Iodine Binding

The structure of the carbohydrate polymers produced by the amylosucrase enzyme can potentially be identified by developing a method to enumerate the proportions of amylose and amylopectin in plant material. The comparison of control samples with samples expressing the amylosucrase gene could identify structural composition changes that may be present in the polymers produced by amylosucrase expressing events, suggesting that a carbohydrate polymer lock is being produced. One possible method for accomplishing this is through an iodine binding assay. In this assay, the plant produced carbohydrate polymers are solubilized from the tissue and then stained with iodine. The resulting iodine-starch complexes will absorb at different wavelengths depending on the proportions of amylose and amylopectin present in the extract. Through comparison with known standards and mixtures of amylose and amylopectin, both the total amount of starch present and the proportions of amylose and amylopectin present in the starch produced in the tissue can be calculated.

The following is an example of a starch extraction and iodine staining procedure that could be used to analyze lyophilized, ground tissue samples. Approximately 100-200 mg of ground, lyophilized tissue should be washed with 5 mL of 90% ethanol, incubated for 15 minutes in a 100 degree C. water bath, and centrifuged for 5 minutes at 3000 rpm to remove the supernatant. This wash step should be repeated at least two more times to ensure sufficient removal of soluble sugars and other potential iodine binding compounds from the samples. To the sample material, 5 mL of 100% ethanol should be added and incubated again for 15 minutes at 100 degree C. Prior to centrifuging the sample, 5 mL of acetone should be added to the mixture. The pellet should then be suspended once more in 5 mL of acetone to ensure the complete removal of any residual ethanol, centrifuged for 5 minutes at 3000 rpm, and the pellet allowed to dry overnight. To solubilize the starch from the dried pellet, 5 mL of 0.5M KOH should be added and incubated for 2-3 hours at 100 degree C. Debris may be pelleted by centrifugation for 10 min at 3000 rpm. For the staining of the solubilized carbohydrate polymers, 1 mL of the KOH extract should first be neutralized with 5 mL of 0.1M HCl, then 0.5 mL of Lugol's Iodine solution should be added and diluted to between 25 and 50 mL with water to bring the absorbance into an appropriate range. The color should be allowed to develop for about 15 minutes and then samples can be added to a microtiter plate for measuring the optical density along with pure amylose and pure amylopectin stained standards. The spectra of the samples and standards should be measured first to determine at which wavelength the maximum absorbance occurs for each sample, since this is indicative of the proportions of amylose and amylopectin in the samples. To analyze the sample spectra, a system of equations will be set up using

Beer's law based on the absorbance values at 6 different wavelengths. Measurements of the absorbance will be recorded at 504 nm, the wavelength of greatest difference between the amylose and amylopectin peaks where amylopectin's absorbance is greater than amylose's absorbance; 548 nm, the wavelength of the pure amylopectin peak; 630 nm, the wavelength of the pure amylose peak; 700 nm, the wavelength of greatest difference between the amylose and amylopectin peaks where amylose's absorbance is greater than amylopectin's absorbance; 800 nm, the wavelength of greatest absorbance due to amylose where amylopectin's absorbance approaches zero; and the wavelength determined to be the location of the sample spectra's maximum (Jarvis and Walker J. Sci. Food Agric. 63: 53-57 (1993)). The results of this system of equations will give a concentration value of the amount of amylose and the amount of amylopectin present in the sample extract, from which a ratio of the two starch forms can be determined.

Upon successful completion of the iodine binding assay, it is expected that the assay data will support the total starch assay data in showing an overall starch increase in the samples expressing the amylosucrase gene. In addition, it is expected that the amylosucrase expressing events will produce a carbohydrate polymer that is more closely related to amylose than amylopectin, therefore a larger proportion of amylose when compared to control samples should be observed. This shift in composition of the starch produced in amylosucrase expressing events will also support the successful production of a locked substrate in plant tissue.

6C: Digestion of Plant Produced Carbohydrate Polymers with Plant-Expressed Enzymes

The ability of a plant produced key enzyme to digest a plant produced locked substrate can be exemplified using the principle underlying the glucose oxidase-peroxidase (GOPOD) reaction. If the plant purified key enzyme acts on the plant produced locked sugar, glucose monomers should be liberated from the locked sugar which can be enumerated by the GOPOD reaction. In order to complete this digestion, however, an appropriate plant expressed key enzyme must be purified and a carbohydrate polymer produced by the amylosucrase enzyme must be solubilized in an appropriate buffer. Alpha-amylase can be collected from transgenic maize plants expressing alpha-amylase in the seed through laboratory established FPLC methods yielding a purified plant-expressed key enzyme (alpha-amylase). Locked sugars produced in tobacco or another plant system by the amylosucrase gene can be extracted in boiling water from lyophilized plant material after washing with 80-90% ethanol to remove any soluble sugars or compounds (Spoehr and Milner J. Biol. Chem. 111 (3): 679-687. (1935)). The alpha-amylase will not yield strictly glucose in its digest, the amount of glucose produced should be sufficient to be detected by the GOPOD reaction assay when compared to a control sample of the undigested locked sugar. It is expected that a difference in glucose levels would be detected in this type of digestion assay, verifying that plant expressed key enzymes are, indeed, capable of digesting plant produced locks.

Additionally, in the process of performing HPSEC on debranched amylosucrose polymer mixture, sample fractions could be collected, and a plant expressed alpha amylase or glucoamylase key enzyme could be used to hydrolyze the starch in the collected fractions to glucose. A GOPOD reaction assay could be used to detect the glucose liberated from the amylosucrose locked-carbohydrate fraction.

6D: Detection of Amylosucrase Activity in Stably Transformed Plants or Plants Transiently Expressing Amylosucrase.

Amylosucrase may be expressed either transiently or through stable transformation of maize, cane, beets, tobacco or other plants with a promoter that drives expression in the appropriate target tissue (leaf, endosperm, embryo, etc.) and with targeting sequences that direct the amylosucrase to the desired subcellular location (vacuole, chloroplast, cytoplasm, apoplast, etc.). A variety of techniques may be used to detect the activity of the amylosucrase gene in plants.

For instance, plant tissue samples expressing the amylosucrase polypeptide may be incubated in the dark for 24 to 48 hours in order for transient starch produced in the chloroplast to be broken down by the plant. Leaf or other tissue may be excised from the plant and dipped into boiling water for one minute to heat kill the tissue. After heat killing plant tissue samples may be incubated in hot ethanol to remove the chlorophyll, repeated washing with hot ethanol may be necessary to remove all the chlorophyll. Once the chlorophyll has been removed, the tissue can be rinsed with cold water and placed on a petri dish. Lugol's solution (5 g iodine (I₂) and 10 g potassium iodide (KI) mixed with 85 ml distilled water), may then be poured over the sample and allowed to incubate at room temperature. Control samples that have been in the dark for 24 hours should contain no starch and should not stain black in Lugol's solution. Samples expressing the amylosucrase gene should stain black due to starch that is produced in the vacuole or other organelles targeted for expression of the Amylosucrase enzyme.

Leaves contain a variety of unique cell types such as the pavement cells that are highly specialized cells making up the majority of the leaf surface. These are easily identified by their puzzle piece shapes (in dicots) and are only found at the leaf surface. They contain no chloroplasts or amyloplasts, so if pavement cells are found to have what appeared to be dark staining "amyloplasts" and these are not observed in pavement cells from "vector only" controls, this would be good evidence that the construct is working and that starch is being produced.

6E: Analysis of Locked Amylosucrose Carbohydrates by HPSEC

Another means of analyzing structural composition changes that may be present in the polymers produced by amylosucrase expressing events is by the use of High-performance size exclusion chromatography, HPSEC. Using HPSEC, a locked amylosucrase carbohydrate polymer could be identified and characterized based on its molecular weight or chain length distribution.

The extraction of starch from plant material for analysis by HPSEC could be carried out essentially as described by Santacruz et al J. Agric. Food Chem. 2004, 52 (7): 1985-1989. Starch could be extracted from plant material such as leaf or callus by lyophilizing and grinding plant material. Powdered lyophilized plant tissue could be mixed with 90% ethanol (v/v) and placed in a boiling water bath for 15 minutes. After centrifugation at 1000 g for 10 minutes, the pellet could be washed three more times with hot 90% ethanol. The pellet can be washed again with 100% ethanol, boiled for 15 minutes. After centrifugation, the supernatant can be discarded and the pellet washed further with acetone, centrifuged and supernatant discarded. The pellet can be dried overnight at room temperature. The dried plant material can be further extracted by addition of 0.2% EDTA to the dried residual pellet and mixed overnight with shaking at room temperature. After centrifugation, the resulting starch pellet can be further extracted by addition of 90% ethanol and boiled for 30 min-

utes. After centrifugation, the supernatant can be saved and the pellet extracted again with 90% ethanol. The supernatants can be combined and mixed with 100% ethanol in a ratio of 1 part DMSO to 9 parts ethanol. The solution can be incubated at room temperature for 15 minutes, centrifuged to obtain a starch pellet. The starch pellet can then be solubilized in 90% DMSO with boiling for 15 minutes. The starch could be done debranched for GPC analysis essentially as described by Yao et al Carbo. Research. 2005, 340:701-710. Debranching of starch can be carried out in a 50 mM Sodium Acetate, pH 4.0 buffer which has been warmed to 42-SOC. A reaction which combines 880 ul of warm NaAc buffer, 120 ul of the DMSO solubilized starch pellet can be prepared. To keep the starch solubilized, the reaction can be heated to 100 C for 10 minutes and then cooled to 22-42 C before addition of 1 U/ml of isoamylase (Megazyme Inc., Ireland.) The digestion reaction can be incubated at 37-42 C with constant agitation for 16-24 hours. After digestion, the debranching reaction can be heated in a boiling water bath for 10 minutes. The starch dispersion can then be concentrated in a Speed-Vac vacuum evaporator. Gel permeation chromatography or HPSEC could be carried out on this concentrated starch sample to characterize the starch structure of the locked amylosucrose carbohydrate. Starch samples can be diluted up to 30 fold in DMSO in preparation for analysis by the HPSEC system.

Using an HPSEC system such as a Waters Breeze 717 system. 50 ul of debranched starch polymer could be injected into a Ultrahydrogel-6x40 mm Guard column (WAT 011565) and Ultrahydrogel 250 A—7.8x300 mm column (WAT011525) with Waters 1515 isocratic HPLC pump and a differential refractometer such as Waters Model 410 for detection. A flow rate of 0.5 mL/min at a column, column temperature of 35 C and detector temperature of 40 C may be used. The molecular weight standards for column calibration could be maltotriose (Sigma), maltoheptose (Sigma), and pullulan standards (P-5, MW 5800; P-10, MW 12,200; P-20, MW 23,700; P-50, MW 48,000, from Shodex, Japan). On the chromatogram the differential refractive index (DRI) value on the y-axis will be the mass response to the carbohydrate at a particular retention time (RT).

Within the separation range of the HPSEC media, the RT on the x-axis will be approximately proportional to the logarithm of the molecular weight (or chain length), and using standards the precise relationship may be determined to generate a standard curve. In this way, the chain length of an amylosucrose polymer may be determined and characterized.

EXAMPLE 7

Transgenic Plants Expressing Key Enzymes

7A: Transient Transgenic Tobacco and Sugar Beet Expressing alpha-1,6-glucosidase

Tobacco and sugar beet leaves transiently expressing an alpha-1,6-glucosidase enzyme were generated essentially as described in Example 3D. Leaves transiently expressing alpha-1,6-glucosidase were generated using the binary vector 902525 or the BCTV binary vector 902526. Both of the binary vectors contain expression cassettes encoding an alpha-1,6-glucosidase (SEQ ID NO: 11) which has been targeted through the ER and is expected to accumulate in the apoplast. Infiltrated tobacco and sugar beet leaves were harvested, extracted and enzyme activity assayed essentially as described in Example 3G. The key enzyme, alpha-1,6-glucosidase, catalyzes the conversion of isomaltulose to the fermentable sugars fructose and glucose and was assayed at 60 degrees C. Carbohydrate analysis of the final filtrate was

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performed using the Dionex system essentially as described in Example 1G. Tables 16-17 outline data demonstrating transient expression of an alpha-1,6-glucosidase in tobacco and sugar beet leaves.

TABLE 16

Carbohydrate analysis of tobacco leaves transiently expressing an alpha-1,6-glucosidase enzyme (SEQ ID NO: 11). Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
sample	Glucose (% total sugar)	Fructose (% total sugar)	Isomaltulose (% total sugar)
902525 binary	11.97	12.46	-24.43
902526 BCTV	22.66	26.95	-49.61
Negative control	-1.67	3.75	-2.08

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control is tobacco leaves transiently expressing a binary vector containing an origin of replication from beet curly top.

TABLE 17

HPAEC analysis of carbohydrate products from sugar beet leaves transiently expressing an alpha-1,6-glucosidase enzyme (SEQ ID NO: 11). Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
sample	Glucose (% total sugar)	Fructose (% total sugar)	Isomaltulose (% total sugar)
902525 binary	19.73	19.10	-38.83
902526 BCTV	14.05	11.91	-25.96
Negative control	6.14	6.61	-12.74

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control is sugar beet leaves transiently expressing a binary vector containing an origin of replication from beet curly top.

7B: Transgenic Maize Callus Expressing alpha-1,6-glucosidase

Transgenic maize callus expressing an alpha-1,6-glucosidase enzyme was generated by bombarding maize embryos with linear polynucleotide sequence. The method of embryo transformation and generation of callus was essentially as described in Example 3F; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic maize cells by growth on mannose. The second polynucleotide sequence, 902435 or 902425, contained a maize optimized polynucleotide sequence encoding an alpha-1,6-glucosidase (SEQ ID NO: 54 or SEQ ID NO: 56). The alpha-1,6-glucosidase was targeted to the endoplasmic reticulum (902435) or to the chloroplast (902425).

Analysis of alpha-1,6-glucosidase enzyme activity in transgenic maize calli was performed by extracting the enzyme from the transgenic calli and incubating the extract with isomaltulose. If alpha-1,6-glucosidase enzyme activity is present, the isomaltulose is converted to glucose and fructose. Essentially, maize calli expressing the alpha-1,6-glucosidase were collected 8 calli per well in Slicprep 96 device. Samples were frozen at -80 degrees C. and thawed at room temperature. Thawed samples were centrifuged at 1770xg and flow-through extract collected. Extracts were heated at 60

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degrees C. for 10 minutes. Extracts were centrifuged at 1770xg 30 minutes at 4 degrees C. to pellet denatured proteins in samples. Equal volumes of clarified extract and reaction buffer (200 mM Isomaltulose, 100 mM HEPES, 0.04% Tween-20, 4 mM EDTA, 40 mM NaOH, 2x protease inhibitor [Roche Complete EDTA-free]) were combined and incubated at 60 degrees C. in BioRad Tetrad 2 thermocycler. Samples were collected at times 0 and 24 hours. Collected samples were incubated at 95 degrees C. for 5 minutes before freezing at -20 degrees C. Samples were analyzed by Dionex. Table 18 outlines data which demonstrates that transgenic maize callus expresses an active alpha-1,6-glucosidase enzyme.

TABLE 18

HPAEC analysis of carbohydrate products from transformed maize callus tissue expressing alpha-1,6-glucosidase enzymes. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
Sample	Glucose (% total sugar)	Fructose (% total sugar)	Isomaltulose (% total sugar)
902435 ER	14.28	18.03	-32.31
902425 (plastid)	7.24	9.26	-16.50
Negative control	0.49	-0.18	-0.31

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control is maize callus transformed with a vector that contains the PMI selectable marker only.

7C: Transgenic Sugarcane Callus Expressing alpha-1,6-glucosidase

Transgenic sugarcane callus expressing an alpha-1,6-glucosidase enzyme was generated essentially as described in Example 3A; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic sugarcane cells by growth on mannose. The second polynucleotide sequence, 902425, contained a polynucleotide sequence encoding an alpha-1,6-glucosidase (SEQ ID NO: 56). The alpha-1,6-glucosidase was targeted to the chloroplast.

Sugarcane calli expressing the alpha-1,6-glucosidase were collected 1 callus per well in 96-well 2 mL plates (Whatman) containing one 3/16" chrome ball bearing per well. The plate was shaken at setting 9 for 2 min in a Kleco Titer plate/Microtube Grinding Mill creating a powder. Buffer (100 mM HEPES, 4 mM EDTA, 0.04% Tween-20, pH 7) was added to the powdered samples to give a thick slurry. Samples were incubated in a Glas-Col rotator at 80% speed for 30 min. Samples were transferred by wide-bore P200 pipet to a 96 well PCR at 100 uL per well and incubated at 60 degrees C. for 20 minutes. Extracts were centrifuged at 1770xg for 30 mins to pellet denatured proteins in samples. Equal volumes of clarified extract and 271 mM trehalulose/134 mM isomaltulose were combined and incubated at 60 degrees C. in BioRad Tetrad 2 thermocycler. Samples were collected at times 0 and 24 hours. Collected samples were incubated at 95 degrees C. for 5 minutes before freezing at -20 degrees C. Samples were analyzed by HPAE chromatography essentially as described in Example 1G. Table 19 demonstrates that sugarcane callus expresses an active alpha-1,6-glucosidase that also shows alpha-1,1-glucosidase activity.

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TABLE 19

Carbohydrate analysis (HPAE chromatography) of products from transformed sugarcane callus tissue expressing an alpha-1,6-glucosidase enzyme. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.				
Sample	Glucose (% total sugar)	Fructose (% total sugar)	Isomaltulose (% total sugar)	Trehalulose (% total sugar)
902425 (plastid)	8.98	9.59	-6.86	-9.60
Negative control	2.53	3.70	-2.82	-2.15

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control is wildtype sugarcane callus.

7D: Transient Expression of alpha-1,1-glucosidase (SEQ ID NO: 27) Enzyme in Sugar Beet or Tobacco Leaves

Tobacco and sugar beet leaves transiently expressing an alpha-1,1-glucosidase (SEQ ID NO: 27) enzyme were generated essentially as described in Example 3D. The vector for transient expression was 901612 or 902522 which are described in Example 12. The binary vector 901612 contains an expression cassette encoding an alpha-1,1-glucosidase (SEQ ID NO: 27) targeted to the chloroplast. The binary vector 902522 contains an expression cassette encoding an alpha-1,1-glucosidase (SEQ ID NO: 27) targeted to pass through the endoplasmic reticulum and accumulate in the apoplast. Infiltrated tobacco and sugar beet leaves were harvested, extracted and enzyme activity assayed essentially as described in Example 3G. The key enzyme, alpha-1,1-glucosidase, catalyzes the conversion of isomaltulose or trehalulose to the fermentable sugars fructose and glucose and was assayed at 70 degrees C. Carbohydrate analysis of the final filtrate was performed using the Dionex system essentially as described in Example 1G. Tables 20-21 outline data demonstrating transient expression of an alpha-1,1-glucosidase in tobacco and sugar beet leaves.

TABLE 20

HPAEC analysis of carbohydrate products from tobacco leaves transiently expressing an alpha-1,1-glucosidase enzyme. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.				
Sample	Glucose (% total sugar)	Fructose (% total sugar)	Trehalulose (% total sugar)	Isomaltulose (% total sugar)
901612	21.61	23.38	-22.57	-22.41
Negative control	1.47	1.55	1.93	-4.95

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control is tobacco leaves transiently expressing empty binary vector.

TABLE 21

HPAEC analysis of carbohydrate products from sugar beet leaves transiently expressing alpha-1,1-glucosidase enzymes. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.				
sample	Glucose (% total sugar)	Fructose (% total sugar)	Trehalulose (% total sugar)	
901612 chloroplast	12.48	13.70	-13.59	

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TABLE 21-continued

HPAEC analysis of carbohydrate products from sugar beet leaves transiently expressing alpha-1,1-glucosidase enzymes. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
sample	Glucose (% total sugar)	Fructose (% total sugar)	Trehalulose (% total sugar)
902522	18.73	19.51	-22.46
apoplast Negative control	6.94	7.45	-5.49

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control is sugar beet leaves transiently expressing empty binary vector

7E: Transgenic Maize Callus Expressing alpha-1,1-glucosidase

Transgenic maize callus expressing alpha-1,1-glucosidase enzyme was generated by bombarding maize embryos with two binary vectors. The method of embryo transformation and generation of callus was essentially as described in Example 3F; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic maize cells by growth on mannose. The second polynucleotide sequence, 902429, contained a maize optimized polynucleotide sequence encoding an alpha-1,1-glucosidase (SEQ ID NO: 49). The alpha-1,1-glucosidase was targeted to be retained by the endoplasmic reticulum.

Maize calli expressing the alpha-1,1-glucosidase was collected 1 callus per well in 96-well 2 mL plates (Whatman) containing one 3/16" chrome ball bearing per well. The plate was shaken at setting 9 for 2 min in a Kleco Titer plate/Microtube Grinding Mill. Sets of 4 pulverized callus tissue samples were combined and transferred to microfuge tubes. The samples were centrifuged at 20,000xg 30 minutes at 4 degrees C. The supernatants containing protein extract were transferred to new tubes and extracts with volumes <20 uL were pooled such that all samples were >30 uL in volume. Equal volume of extract and reaction buffer (~185 mM trehalulose, 93 mM isomaltulose, 100 mM HEPES, 0.04% Tween-20, 4 mM EDTA, 40 mM NaOH, Roche protease inhibitors) were combined and incubated at 70 degrees C. in BioRad Tetrad 2 thermocycler. Samples were collected at times 0 and 24 hours. Collected samples were incubated at 95 degrees C. for 5 minutes before freezing at -20 degrees C. Samples were analyzed by Dionex essentially as described in Example 1G. Table 22 demonstrates that maize callus expresses an active alpha-1,1-glucosidase.

TABLE 22

HPAEC analysis of carbohydrate products from transformed maize callus tissue expressing an alpha-1,1-glucosidase enzyme. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
Sample	Glucose (% total sugar)	Fructose (% total sugar)	Trehalulose (% total sugar)
902429	10.02	11.32	-6.47
Negative control	3.51	3.46	1.50

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control was transgenic maize callus generated by transformation with the binary vector expressing the selectable marker (PMI) only.

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7F: Transient Expression of alpha-1,5-glucosidase by Tobacco Leaves

Tobacco leaves transiently expressing an alpha-1,5-glucosidase (SEQ ID NO: 46) enzyme were generated essentially as described in Example 3D. The vector for transient expression was BCTV binary vector 902550 which is described in Example 12. BCTV binary vector 902550 contains an expression cassette encoding an alpha-1,5-glucosidase (SEQ ID NO: 46) which is targeted to the chloroplast. Infiltrated tobacco and sugar beet leaves were harvested, extracted and enzyme activity assayed essentially as described in Example 3G. The key enzyme, alpha-1,5-glucosidase, catalyzes the conversion of leucrose to the fermentable sugars glucose and fructose and was assayed at 80 degrees C. Table 23 outlines data demonstrating tobacco leaves transiently expressed the alpha-1,5-glucosidase enzyme.

TABLE 23

HPAEC analysis of carbohydrate products from tobacco leaves transiently expressing an alpha-1,5-glucosidase enzyme. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
sample	Glucose (% total sugar)	Fructose (% total sugar)	Leucrose (% total sugar)
902550	18.07	20.36	-38.43
Negative control	3.30	1.50	-4.80

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. The negative control is tobacco leaves transiently expressing empty BCTV vector.

7G: Transgenic Maize Callus Expressing alpha-1,5-glucosidase (SEQ ID NO: 43)

Transgenic maize callus expressing alpha-1,5-glucosidase enzyme was generated by bombarding maize embryos with two binary vectors. The method of embryo transformation and generation of callus was essentially as described in Example 3F; however, two polynucleotide sequences were bombarded at the same time. One of the polynucleotide sequences contained the selectable marker, PMI, which allows for selection of transgenic maize cells by growth on mannose. The second polynucleotide sequence, 902423, contained a maize optimized polynucleotide sequence encoding an alpha-1,5-glucosidase (SEQ ID NO: 43). The alpha-1,5-glucosidase was targeted to the chloroplast.

Maize calli expressing an alpha-1,5-glucosidase (SEQ ID NO: 43) was collected 1 callus per well in 96-well 2 mL plates (Whatman) containing one 3/16" chrome ball bearing per well. Samples were frozen at -80 degrees C. The frozen material was shaken at setting 9 for 4 min in a Kleco Titer plate/Microtube Grinding Mill. 200 uL of extraction buffer (100 mM HEPES, 4 mM EDTA, 0.04% Tween-20, pH 7) was added to each sample. Extracts were incubated in a Glas-Col rotator at 80% speed for 10 min. Extract was centrifuged at 1770xg for 10 minutes at 4 degrees C. in Eppendorf 5810R swing bucket centrifuge. Extract was frozen at -80 degrees C. Extract was later thawed and transferred to a 96-well PCR plate (Thermo Sci). Samples were heated at 80 degrees C. for 15 minutes in BioRad Tetrad 2 thermocycler. Plates were again centrifuged at 1770xg for 10 minutes at 4 degrees C. in Eppendorf 5810R swing bucket centrifuge. Supernatants were filtered using a Millipore Multiscreen-HV filter plate. Filtered extracts of 8 callus samples were combined. Combined samples were concentrated from ~1.6 mL to 100-500 uL using Microcon concentrators with MWCO 3 k membrane

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filters (Amicon). An equal volume of 200 mM leucrose and extract was added to 96-well PCR plate and incubated at 80 degrees C. in the thermocycler. Samples were collected at times 0 and 24 hours. Collected samples were incubated at 95 degrees C. 5 minutes before freezing at -20 degrees C. Samples were analyzed by Dionex essentially as described in Example 1G. Alpha-1,5-glucosidase activity was confirmed by measuring the conversion of the locked sugar, leucrose, to the fermentable sugars glucose and fructose. Table 24 demonstrates that maize callus expressed an active alpha-1,5-glucosidase enzyme.

TABLE 24

HPAEC analysis of carbohydrate products from transformed maize callus tissue expressing an alpha-1,5-glucosidase enzyme. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.			
sample	Glucose (% total sugar)	Fructose (% total sugar)	Leucrose (% total sugar)
902423	6.86	12.71	-19.57
Negative control	0.48	0.73	-1.21

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control consisted of maize callus transformed with the binary vector containing the selectable marker (PMI) only.

EXAMPLE 8

Combining Plant Expressed Locking and Key Enzymes

Tobacco leaves transiently expressing enzymes were generated essentially as described in Example 3D. Leaves were generated by transiently expressing two binary vectors simultaneously. One of the binary vectors was 17588 (described in Example 12) which contains a polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 16). The second binary vector was 902526 (described in Example 12) which contains a polynucleotide sequence encoding an alpha-1,6-glucosidase (SEQ ID NO: 11). Both binary vectors were infiltrated into the same tobacco leaf.

Essentially as described in Example 3D, whole leaves from tobacco were co-infiltrated with both binary vectors 17588 and 092526. Co-infiltration was performed essentially as described in Example 3D except that two strains of *Agrobacterium*, each containing one of the two vectors, were infiltrated into the tobacco leaf. Infiltrated leaves were collected and frozen at -80 degrees C. in 24-well blocks containing 3/16" chrome ball bearings. The frozen material was shaken at setting 9 for 2 min in a Kleco Titer Plate/Microtube Grinding Mill creating a powder. Powder samples were transferred to 30 mL centrifuge tubes and centrifuged at 20,000xg for 20 minutes at 4 degrees C. The supernatants were transferred to new tubes and adjusted to 50 mM HEPES, 0.02% Tween-20, 2 mM EDTA and 20 mM NaOH resulting in a mixture with pH between 7 and 8. Samples were then transferred to PCR tubes and incubated at 60 degrees C. in a Biorad Tetrad 2 thermocycler. Samples were collected from the thermocycler at times 0, 18, and 48 hours and heated at 95 degrees C. before freezing at -20 degrees C. The sugar contents of the samples thawed after the -20 degree C. freeze were analyzed by Dionex.

Table 25 demonstrates that plants transiently expressing both sucrose isomerase and alpha-1,6-glucosidase expressed an active sucrose isomerase. Sucrose isomerase activity was

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demonstrated by the accumulation of trehalulose and isomaltulose in both the negative control (17588) and the sample (17588 and 902526). It is noted that the sample (17588 and 902526) accumulated less trehalulose and isomaltulose than the negative control (17588). While not to be limited by theory, this observation suggests that the alpha-1,6-glucosidase enzyme is active in the sample (17588 and 902526) and thus leads to the conversion of the trehalulose and isomaltulose to fermentable sugars.

Tables 25-26 demonstrate that plants transiently expressing both sucrose isomerase and alpha-1,6-glucosidase expressed active enzymes. Alpha-1,6-glucosidase activity was demonstrated by comparing time 0 samples with samples collected at 48 hours which demonstrated the conversion of the locked sugars, trehalulose and isomaltulose, to the fermentable sugars, glucose and fructose.

Data outlined in Table 25-26 demonstrates the co-expression of a locking enzyme (sucrose isomerase) and an key enzyme (alpha-1,6-glucosidase) in a plant.

TABLE 25

HPAEC analysis of carbohydrate products from tobacco leaves transiently expressing both sucrose isomerase and an alpha-1,6-glucosidase enzyme. Accumulation of sucrose isomers in a plant co-expressing both lock and key enzymes before incubating for key activity. (<i>T. ethanolicus</i>)				
sample	Glucose + Fructose % total sugar	Sucrose % total sugar	Trehalulose % total sugar	Isomaltulose % total sugar
17588 and 902526	75.88	0	15.91	8.21
Negative control	80.99	19.01	0	0

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control consisted of non-infiltrated tobacco leaves.

TABLE 26

HPAEC analysis of carbohydrate products from tobacco leaves transiently expressing both sucrose isomerase and an alpha-1,6-glucosidase enzyme. Table 254 converts hydrolysis of the lock sugars by key activity after incubation. Enzyme activity is indicated by the change in abundance of each sugar as a percentage of the total sugars over a 24 hour period.				
sample	Glucose (% total sugar)	Fructose (% total sugar)	Isomaltulose (% total sugar)	Trehalulose (% total sugar)
17588 and 902526	0.15	10.34	-4.20	-6.30
Negative control	-8.18	3.58	1.19	3.41

Total sugar = total amount of identifiable sugars in sample based on retention times of pure sugar standards. Extraneous peaks in samples are indeterminate and not included in sample analysis. Negative control consisted of tobacco leaves transiently expressing sucrose isomerase and an empty control vector.

EXAMPLE 9

Production of Fermentable Sugars and/or Ethanol

9A: Glucose Production Using Both Dextranucrase and Dextranase

Dextranucrase and dextranase form a pair of enzymes that are a lock and key combination. The dextranucrase catalyzes the formation of dextrans which are a locked form of sugar or carbohydrate. The dextranase is a key enzyme which can be used to convert the dextran back to a fermentable form of sugar.

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The dextranucrase is expressed in transgenic sugarcane plants such that dextrans accumulate in the sugarcane plant. Dextrans produced from dextranucrase reactions in sugarcane juice (Example 1C) or dextrans produced by transgenic plants expressing dextranucrases (Example 3B) are harvested. These dextrans are used as substrate for dextranase activity assays to demonstrate the ability of the selected dextranases to convert the dextrans back into glucose, maltose and other small reducing sugars. The dextranase is provided as either transgenic plant produced enzyme (Example 3C) or as microbially produced enzyme (Example 2C).

9B: Isomaltulose Fermented to Produce Ethanol

Yeast, *Saccharomyces cerevisiae*, strains were screened for the ability to ferment isomaltulose into ethanol. Strains were grown in a media containing 10 g yeast extract, and 20 g peptone per liter of media. This media was supplemented with glucose or isomaltulose to the appropriate final concentration.

Single yeast colonies were inoculated into 5 mL 2% glucose media and incubated for 24 hours at 30 degrees C. cells were centrifuged at 3000xg for 5 minutes, supernatant was discarded, cells were washed by resuspending the cells in 5 mLs of distilled water, washed cells were centrifuged at 3000xg for 5 minutes, supernatant was discarded, cells were resuspend in 5 mLs of yeast media containing 1% isomaltulose media and incubated for 12 hours at 30 degrees C. After 12 hours cells were centrifuged at 3000xg for 5 minutes, supernatant was discarded, cells were washed by resuspending in 5 mLs of distilled water, washed cells were centrifuged at 3000xg for 5 minutes, supernatant was discarded, cells were resuspend in 5 mLs of 4% isomaltulose media or 4% glucose media for fermentation. Samples for ethanol and sugar analysis were removed every hour for six hours and stored at -20 degrees C. After all samples were collected they were thawed and filtered in 0.45 Micron nylon SpinX columns by centrifugation at 7000 rpm for 5 minutes. Filtered solution was then subjected to HPLC to determine the concentration of ethanol and the sugar composition of the solution which is shown in table 27. The graph below outlines the ethanol produced by various yeast strains grown in the presence of glucose or isomaltulose over time.

TABLE 27

Ethanol yield from yeast strains grown with isomaltulose or glucose			
Yeast Strain	Sugar	Percentage Ethanol Yield	Percentage of Theoretical Yield
B	Glucose	2.1	80.1
B	Isomaltulose	1.49	57.4
C	Glucose	2.14	82.0
C	Isomaltulose	0.35	13.6
A	Glucose	1.9	72.4
A	Isomaltulose	0	

EXAMPLE 10

Transfer of Ethanol Producing Genes Between Yeast Strains

Not all yeast strains, including commercial yeast strains used in the ethanol industry, possess the capacity for isomaltulose fermentation. Genes needed for isomaltulose fermentation can be introduced into commercial strains by mating, mutagenesis or transformation. These genes may include an alpha glucosidase enzyme in addition to a receptor which

senses the presence of isomaltulose and induces the expression of an alpha-glucoside transporter which transports isomaltulose and other alpha glucosides into the cell. Genes involved with these functions occur at the melezitose locus in *S. cerevisiae* and may be introduced into other strains of yeast by mating techniques known to skilled practitioners in the art (Hwang & Lindegren Nature vol 203 no 4946, pp 791-792 (1964)). Alternatively, the coding sequence of a highly efficient alpha-1,6-glucosidase enzyme may be introduced into yeast in place of the alpha glucosidase gene at the melezitose locus by homologous recombination or they may be inserted elsewhere in the genome. By replacing the endogenous alpha-glucosidase gene with a gene that more efficiently hydrolyzes isomaltulose or other locked sugars it may be possible to improve the rate of fermentation of these sugars. Similarly, genes for alpha-glucoside transporters and receptors may be overexpressed or altered by site directed mutagenesis in order to increase the rate of isomaltulose uptake by yeast strains to improve the efficiency of isomaltulose fermentation. Another approach may be to identify strains which constitutively express the genes necessary for isomaltulose fermentation or to mutagenize or engineer yeast strains so that they constitutively express the genes necessary for isomaltulose fermentation. The techniques necessary for these approaches are widely known to skilled practitioners of the art.

10A: Transgenic Yeast Expressing Key Enzymes

A yeast codon optimized gene for *Bacillus* SAM1606 (Sc_SAM1606) glucosidase (GeneBank Accession CAA54266) was cloned into the XhoI/XbaI sites of pGEM30 (ATCC 53345), which contains an N-terminus DEX4 secretion signal. This created a DEX4-Sc_SAM1606 glucosidase fusion protein.

The URA3 marker was replaced with the kanMX locus, which confers resistance to the antibiotic Geneticin (G418) (Wach et al. Yeast 10: 1793-1808 (1994)). The URA3 cassette was excised with SmaI and ClaI and the backbone was gel-purified. The kanMX cassette was amplified from a yeast insertional library (ATCC number GSA-7) using Phusion High Fidelity DNA polymerase (Finnzymes) with primers bearing 30 bp of homology to the ends of the SmaI/ClaI backbone fragment.

The SmaI/ClaI backbone fragment and the kanMX cassette were recombined using SLIC recombination (Li and Elledge, Nature Methods 4: 251-256 (2007)). Briefly, both fragments were treated with T4 DNA polymerase at room temperature to create single stranded DNA, the reaction was stopped after 15 minutes with dCTP, and the fragments were co-transformed into *E. coli* TOP10 competent cells (Invitrogen). Plasmids isolated from recombinant *E. coli* cells were sequenced and analyzed by restriction enzymes. The resulting vector was named pEB68.

A second yeast vector containing the *Bacillus thuringiensis* alpha-1,6-glucosidase gene was generated by cloning a yeast codon optimized polynucleotide sequence encoding the alpha-1,6-glucosidase into the pEB68 backbone by SLIC recombination to create pEB77.

An 'empty-vector' control consisting of the pEB68 backbone but lacking any gene behind the TP1 promoter was made by cutting pEB68 with XhoI/XbaI, purification of the backbone, blunting the ends, and self-ligation. This vector was named pEB70.

Saccharomyces cerevisiae strain X1049-9C (ATCC number 204802) was transformed with the vectors pEB68, pEB77, and pEB70. Yeast competent cells were made and transformed using the S. c. EasyComp™ Transformation kit (Invitrogen). Transformed yeast cells were recovered by

holding them at 30 degreesC for 4-5 hours after transformation and then plated on YPD medium containing 200 ug/mL of G418.

Glucosidase enzyme activity associated with vector pEB69 was measured in transformed yeast cells by selected three yeast clones expressing DEX4-Sc_SAM1606 fusion protein and three untransformed yeast clones which were inoculated on 5 mL of YPD with G418 (untransformed yeast was inoculated in YPD without selection). After 24 hours of growth, cells were pelleted and the media was separated and used for enzyme analyses.

Sc-SAM1606 activity was measured at 70 degreesC for 16 hours by combining 10 uL of yeast media, 25 uL of buffer (100 mM Hepes, 4 mM EDTA, 0.04% Tween-20, pH 7.0), and 15 uL of a sugar solution containing 280 mM trehalulose, 100 mM isomaltulose, 70 mM citrate. Enzyme activity was estimated by measuring the amount of glucose released from the conversion of locked sugar (trehalulose and isomaltulose) to glucose using a GO-POD assay essentially as described in Example 2B. Table 27 outlines data demonstrating the transformed yeast expressed an active glucosidase enzyme.

Glucosidase enzyme activity associated with vector pEB77 was demonstrated by isolating two clones of each transformation (pEB77 and pEB70) and inoculated into medium containing 10 g yeast extract, 20 g peptone, 4 g isomaltulose, and 0.5% glucose per liter of medium. Cultures were grown until glucose was exhausted (24 hours). After 24 hours, the cells were spun and 1 mL of medium was saved for enzyme activity. To evaluate glucosidase activity on isomaltulose the following reaction was set up: 25 ul of 2x Buffer (100 mM Hepes pH: 7.0, 4 mM EDTA, 0.04% Tween-20, protease inhibitors), 10 ul isomaltulose (500 mM), and 15 ul medium obtained as described above. The 50 uL reaction was incubated overnight at 37 degrees C. 20 uL of the above reaction were added to 250 uL of Glucose oxidase reagent (GOPOD assay essentially as described in Example 2B) and incubated at 37 degrees C. for 10 minutes. The reactions consisted of three technical replicates. The glucose concentration measured was termed GlucoseA. To account for any glucose left in the medium after 24 hours of yeast growth, the same GOPOD assay was conducted by diluting 15 uL of medium with 35 uL of water (no isomaltulose) and using 20 uL of this dilution to the Glucose oxidase reagent. All the glucose measured this way is considered background noise and must come from the medium. This was termed GlucoseB.

The amount of glucose produced by hydrolysis of isomaltulose was calculated as GlucoseA minus GlucoseB and correspond to the values shown in Table 29.

TABLE 28

Glucose Conc of samples (mM): Transformed raw data from yeast expressing glucosidase using equation from glucose standard curve.					
Sample #					
Sample Replicate	pEB68	pEB68	pEB68	pEB68	Negative control
A	4.74	7.19	4.21	4.73	1.49
B	4.81	3.86	4.26	4.59	1.65
C	4.83	4.50	4.47	4.90	1.63

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EXAMPLE 11

Improvement of Molecules to Increase Activity,
Thermostability, and Catalytic Efficiency and
Product Specificity

Improvement of sucrose isomerase enzymes can be achieved through rational design of the enzyme. For example, the product of the *pall* gene (GenBank accession number AY040843) contains a product specificity domain³²⁵RLDRD³²⁹ which influences the proportion of trehalulose or isomaltulose produced by the enzyme. By mutating these four charged amino acid residues (Arg325, Arg328, Asp327 and Asp329) trehalulose formation can be increased by 17-61% and formation of isomaltulose can be decreased by 26-67% (Zhang et al. FEBS Letters 534 (2003) 151-155). An aromatic clamp formed by Phe 256 and Phe280 has also been identified as important in substrate recognition and product specificity. (Ravaud et al. The Journal of Biological Chemistry VOL. 282, NO. 38, pp. 28126-28136, Sep. 21, 2007).

EXAMPLE 12

Constructs for Transient Expression

Table 1 outlines expression constructs used for generation of stable, transgenic plants as well as for the expression of enzymes transiently in plant tissues. The DNA sequences encoding proteins were codon optimized for the appropriate host; for example, expression constructs designed for tobacco and sugarbeet transient and stable transgenic plant expression were codon optimized for dicots while expression constructs designed for sugarcane or maize transient and stable transgenic plant expression were codon optimized for monocots. Codon optimization tables are available through commercial software applications such as Vector NTI 9.0.

Standard cloning techniques such as restriction enzyme digestion, gel electrophoresis and subsequent fragment purification, DNA ligation, bacterial cell transformation and selection, and the like were used to generate the vectors described in Table 29. Some of the components of the expression vectors described in Table 1 were synthesized by Gene-Art (Germany), additionally, some of the vectors were cloned by GeneArt (Germany).

The binary vector 17588 contains an expression cassette with the following components operatively linked together in this order: the *Arabidopsis* ubiquitin promoter (SEQ ID NO: 7); GY1 ER targeting sequence (SEQ ID NO: 13), which targets the polypeptide encoded by the sucrose isomerase coding region through the endoplasmic reticulum; the sporamin vacuolar targeting sequence (SEQ ID NO 15) which directs the sucrose isomerase polypeptide from the endoplasmic reticulum to the vacuole; a dicot optimized polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 16); and a NOS termination sequence.

The binary vector pEB47 contains an expression cassette with the following components operatively linked together in this order: an FMV enhancer (SEQ ID NO: 22); a 35S enhancer (SEQ ID NO: 23); a maize ubiquitin promoter (SEQ ID NO: 18); a maize gamma-zein ER targeting sequence (SEQ ID NO: 19) which directs the sucrose isomerase polypeptide to the ER; a sporamin vacuolar targeting sequence (SEQ ID NO: 15) which directs the sucrose isomerase polypeptide from the ER to the vacuole; a maize optimized polynucleotide sequence encoding a sucrose isomerase (SEQ ID NO: 24); a NOS terminator.

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The vector pEB38 contains an expression cassette with the following components operatively linked together in this order: maize ubiquitin promoter (SEQ ID NO: 18); maize gamma zein signal sequence (SEQ ID NO: 19) which targets the polypeptide encoded by the sucrose isomerase polynucleotide sequence to the endoplasmic reticulum; sporamin vacuolar targeting sequence (SEQ ID NO: 15) which directs the polypeptide encoded by the sucrose isomerase polynucleotide sequence from the endoplasmic reticulum to the vacuole; monocot optimized polynucleotide sequence encoding sucrose isomerase (SEQ ID NO: 20); and the NOS terminator.

The binary vector 902525 contains an expression cassette with the following components operatively linked together in this order: *Arabidopsis* ubiquitin promoter (SEQ ID NO: 7); GY1 ER targeting sequence (SEQ ID NO: 13), which targets the polypeptide encoded by the sucrose isomerase coding region through the endoplasmic reticulum; dicot optimized polynucleotide sequence encoding sucrose isomerase polypeptide (SEQ ID NO: 11); NOS terminator. The sucrose isomerase enzyme expressed by this expression cassette is expected to accumulate in the apoplast of the transgenic plant cell comprising the expression cassette.

The BCTV binary vector 902526 contains an expression cassette with the following components operatively linked together in this order: *Agrobacterium* NOS promoter (SEQ ID NO: 10); GY1 ER targeting sequence (SEQ ID NO: 13), which targets the polypeptide encoded by the sucrose isomerase coding region through the endoplasmic reticulum; dicot optimized polynucleotide sequence encoding sucrose isomerase polypeptide (SEQ ID NO: 11); NOS terminator. The sucrose isomerase enzyme expressed by this expression cassette is expected to accumulate in the apoplast of the transgenic plant cell comprising the expression cassette.

The binary vector 901612 contains an expression cassette with the following components operatively linked together in this order: *Arabidopsis* ubiquitin promoter (SEQ ID NO: 7); FNR plastid targeting sequence (SEQ ID NO: 26) which directs the alpha-1,1-glucosidase polypeptide to the chloroplast; dicot optimized polynucleotide sequence encoding alpha-1,1-glucosidase (SEQ ID NO: 27); NOS terminator. The alpha-1,1-glucosidase enzyme expressed by this expression cassette is expected to accumulate in the chloroplast of the transgenic plant cell comprising the expression cassette.

The binary vector 902195 contains an expression cassette with the following components operatively linked together in this order: *Agrobacterium* NOS promoter (SEQ ID NO: 10); GY1 ER targeting sequence (SEQ ID NO: 13) which targets the dextranucrase polypeptide to the endoplasmic reticulum; sporamin vacuolar targeting sequence (SEQ ID NO: 15) which directs the polypeptide encoded by the dextranucrase polynucleotide sequence from the endoplasmic reticulum to the vacuole; dicot optimized polynucleotide sequence encoding a dextranucrase with leucrose synthase activity (SEQ ID NO: 35); NOS terminator.

The vector pEB28 contains an expression cassette with the following components operatively linked together in this order: maize ubiquitin promoter (SEQ ID NO: 18); maize gamma zein signal sequence (SEQ ID NO: 19) which targets the polypeptide encoded by the dextranucrase polynucleotide sequence to the endoplasmic reticulum; sporamin vacuolar targeting sequence (SEQ ID NO: 15) which directs the polypeptide encoded by the dextranucrase polynucleotide sequence from the endoplasmic reticulum to the vacuole; monocot optimized polynucleotide sequence encoding a dextranucrase with leucrose synthase activity (SEQ ID NO: 37); NOS terminator.

The binary vector 902550 contains an expression cassette with the following components operatively linked together in this order: *Arabidopsis* ubiquitin promoter (SEQ ID NO: 7); chloroplast targeting sequence (SEQ ID NO: 42); dicot optimized polynucleotide sequence encoding an alpha-1,5-glucosidase (SEQ ID NO: 46); NOS terminator.

The vector 902423 contains an expression cassette with the following components operatively linked together in this order: maize ubiquitin promoter (SEQ ID NO: 39); TMV enhancer (SEQ ID NO: 40); chloroplast targeting sequence (SEQ ID NO: 41) which directs the alpha-1,5-glucosidase polypeptide encoded by the polynucleotide sequence (SEQ ID NO: 43) to the chloroplast; maize optimized polynucleotide sequence encoding alpha-1,5-glucosidase (SEQ ID NO: 43); terminator from maize ubiquitin (SEQ ID NO: 45).

The binary vector 90522 contains an expression cassette with the following components operatively linked together in this order: *Arabidopsis* ubiquitin promoter (SEQ ID NO: 7); GY1 ER targeting sequence (SEQ ID NO: 13) which targets the alpha-1,1-glucosidase polypeptide to the endoplasmic

reticulum; dicot optimized polynucleotide sequence encoding an alpha-1,1-glucosidase (SEQ ID NO: 52); NOS terminator. The expectation is that the alpha-1,1-glucosidase polypeptide will be processed through the endoplasmic reticulum and accumulate in the apoplast.

The vector 902435 contains an expression cassette with the following components operatively linked together in this order: maize ubiquitin promoter (SEQ ID NO: 29); TMV enhancer sequence (SEQ ID NO: 40); maize optimized polynucleotide sequence encoding an alpha-1,6-glucosidase (SEQ ID NO: 54); ER retention sequence (SEQ ID NO: 51); maize ubiquitin termination sequence (SEQ ID NO: 45).

The vector 902425 contains an expression, cassette with the following components operatively linked together in this order: maize ubiquitin promoter (SEQ ID NO: 29); TMV enhancer sequence (SEQ ID NO: 40); chloroplast targeting sequence (SEQ ID NO: 26); monocot optimized polynucleotide sequence encoding an alpha-1,6-glucosidase (SEQ ID NO: 56); maize ubiquitin termination sequence (SEQ ID NO: 45).

TABLE 29

Expression constructs				
Vector number	Promoter	Regulatory elements	Enzyme	crop
17588 (binary vector)	<i>Arabidopsis</i> ubiquitin promoter (SEQ ID NO: 7)	GY1 ER targeting sequence (SEQ ID NO: 13); sporamin vacuolar targeting sequence (SEQ ID NO: 15)	Sucrose isomerase (SEQ ID NO: 16)	Sugar beet and tobacco
pEB47 (binary vector)	maize ubiquitin promoter (SEQ ID NO: 18)	FMV enhancer (SEQ ID NO: 22); 35S enhancer (SEQ ID NO: 23); Maize ? gamma zein ER targeting sequence (SEQ ID NO: 19); sporamin vacuolar targeting sequence (SEQ ID NO: 15)	Sucrose isomerase (SEQ ID NO: 24)	Maize and sugarcane
pEB38	maize ubiquitin promoter (SEQ ID NO: 18)	Maize gamma zein ER targeting sequence (SEQ ID NO: 19); sporamin vacuolar targeting sequence (SEQ ID NO: 15)	Sucrose isomerase (SEQ ID NO: 20)	Maize and sugarcane
902525 binary	<i>Arabidopsis</i> ubiquitin promoter (SEQ ID NO: 7)	GY1 ER targeting sequence (SEQ ID NO: 13)	<i>T. ethanolicus</i> alpha-1,6-glucosidase (SEQ ID NO: 11)	Sugar beet and tobacco
902526 (BCTV binary)	NOS promoter (SEQ ID NO: 10)	GY1 ER targeting sequence (SEQ ID NO: 13)	<i>T. ethanolicus</i> alpha-1,6-glucosidase (SEQ ID NO: 11)	Sugar beet and tobacco
902195	NOS promoter (SEQ ID NO: 10)	GY1 ER targeting sequence (SEQ ID NO: 13); sporamin vacuolar targeting sequence (SEQ ID NO: 15)	Dextranase (SEQ ID NO: 35)	Tobacco and sugarbeet
pEB28	maize ubiquitin promoter (SEQ ID NO: 18)	Maize gamma zein ER targeting sequence (SEQ ID NO: 19); sporamin vacuolar targeting sequence (SEQ ID NO: 15)	Dextranase (SEQ ID NO: 37)	Maize and sugarcane
902435	maize ubiquitin promoter (SEQ ID NO: 39)	ER retention sequence (51); maize ubiquitin terminator (SEQ ID NO: 45); TMV enhancer (SEQ ID NO: 40)	Alpha-1,6-glucosidase (SEQ ID NO: 54)	Maize and sugarcane
902425	maize ubiquitin promoter (SEQ ID NO: 39)	TMV enhancer (SEQ ID NO: 40); FNR chloroplast targeting sequence (SEQ ID NO: 41); maize ubiquitin terminator (SEQ ID NO: 45)	Alpha-1,6-glucosidase (SEQ ID NO: 56)	Maize and sugarcane

TABLE 29-continued

Expression constructs				
Vector number	Promoter	Regulatory elements	Enzyme	crop
901612	<i>Arabidopsis</i> ubiquitin promoter (SEQ ID NO: 7)	Plastid targeting sequence FNR (SEQ ID NO: 26)	<i>Bacillus</i> alpha-1,1-glucosidase (SEQ ID NO: 27)	Sugar beet and tobacco
902522	<i>Arabidopsis</i> ubiquitin promoter (SEQ ID NO: 7)	GY1 ER targeting sequence (SEQ ID NO: 13)	Alpha-1,1-glucosidase (SEQ ID NO: 52)	Sugar beet and tobacco
902429	maize ubiquitin promoter (SEQ ID NO: 39)	TMV enhancer (SEQ ID NO: 40); ER targeting sequence (SEQ ID NO: 48); ER retention sequence (51); maize ubiquitin terminator (SEQ ID NO: 45)	Alpha-1,1-glucosidase (SEQ ID NO: 49)	Maize and sugarcane
902550	<i>Arabidopsis</i> ubiquitin promoter (SEQ ID NO: 7)	Plastid targeting sequence FNR (SEQ ID NO: 26)	Alpha-1,5-glucosidase (SEQ ID NO: 46)	Sugarbeet and tobacco
902423	maize ubiquitin promoter (SEQ ID NO: 39)	TMV enhancer (SEQ ID NO: 40); FNR chloroplast targeting sequence (SEQ ID NO: 41); maize ubiquitin terminator (SEQ ID NO: 45)	Alpha-1,5-glucosidase (SEQ ID NO: 43)	Maize and sugarcane

The following embodiments are encompassed by the present invention:

1. A method for producing fermentable sugar comprising:
 - a) providing transgenic plant material comprising one or more locked carbohydrates; and
 - b) contacting said transgenic plant material with one or more key enzymes wherein said contacting is under conditions sufficient for conversion of said locked carbohydrate to fermentable sugar.
2. The method of claim 1, wherein the one or more locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextrans, fructans, maltulose, turanose and isomaltose.
3. The method of claim 1, wherein the one or more key enzyme is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.
4. The method of claim 1, wherein the one or more key enzyme is provided by a source selected from the group consisting of transgenic plant material expressing a key enzyme, recombinant microbe expressing a key enzyme, transgenic yeast expressing a key enzyme, microbe expressing a key enzyme and yeast expressing a key enzyme.
5. The method of claim 1, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.
6. A method for producing fermentable sugar comprising:
 - a) providing transgenic plant material comprising one or more lock enzymes and one or more locked carbohydrates; and
 - b) contacting said transgenic plant material with one or more key enzymes wherein said contacting is under conditions sufficient for conversion of said locked carbohydrate to fermentable sugar.
7. The method of claim 6, wherein the one or more locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

8. The method of claim 6, wherein the one or more lock enzymes is selected from the group consisting of dextransucrase, levan sucrose, alternansucrase, sucrose isomerase and amylosucrase.

9. The method of claim 6, wherein the one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

10. The method of claim 6, wherein the one or more key enzymes is provided by a source selected from the group consisting of transgenic plant material expressing a key enzyme, recombinant microbe expressing a key enzyme, transgenic yeast expressing a key enzyme, microbe expressing a key enzyme and yeast expressing a key enzyme.

11. The method of claim 6, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

12. A method for producing alcohol comprising:

- a) providing transgenic plant material comprising one or more locked carbohydrates;
- b) contacting said transgenic plant material with one or more key enzymes wherein said contacting is under conditions sufficient for conversion of said one or more locked carbohydrates to fermentable sugar; and
- c) fermenting said fermentable sugar to form alcohol.

13. The method of claim 12, wherein the locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

14. The method of claim 12, wherein the one or more key enzyme is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

15. The method of claim 12, wherein the one or more key enzyme is provided by a source selected from the group consisting of transgenic plant material expressing a key enzyme, recombinant microbe expressing a key enzyme,

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transgenic yeast expressing a key enzyme, microbe expressing a key enzyme and yeast expressing a key enzyme.

16. The method of claim 12, wherein the alcohol is selected from the group consisting of ethanol and butanol.

17. The method of claim 12, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

18. A method for producing alcohol comprising:

a) providing transgenic plant material comprising one or more lock enzymes and one or more locked carbohydrates;

b) contacting said transgenic plant material with one or more key enzymes wherein said contacting is under conditions sufficient for conversion of said one or more locked carbohydrates to fermentable sugar; and

c) fermenting said fermentable sugar to form alcohol.

19. The method of claim 18, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

20. The method of claim 18, wherein the one or more lock enzymes is selected from the group consisting of dextranucrase, levan sucrose, alternansucrase, sucrose isomerase and amylosucrase.

21. The method of claim 18, wherein the one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

22. The method of claim 18, wherein the one or more key enzymes is provided by a source selected from the group consisting of transgenic plant material expressing a key enzyme, recombinant microbe expressing a key enzyme, transgenic yeast expressing a key enzyme, microbe expressing a key enzyme and yeast expressing a key enzyme.

23. The method of claim 18, wherein the alcohol is selected from the group consisting of ethanol and butanol.

24. The method of claim 18, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

25. A method for producing fermentable sugar comprising:

a) providing transgenic plant material comprising one or more locked carbohydrates and one or more key enzymes; and

b) processing said transgenic plant material under conditions sufficient for one or more key enzymes to convert one or more locked carbohydrates to fermentable sugar.

26. The method of claim 25, wherein the one or more key enzymes is targeted away from the one or more locked carbohydrates.

27. The method of claim 25, wherein the one or more key enzymes is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

28. The method of claim 25, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

29. The method of claim 25, wherein the one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

30. The method of claim 25, wherein the one or more key enzymes is provided by a source selected from the group consisting of transgenic plant material expressing a key enzyme, recombinant microbe expressing a key enzyme, transgenic yeast expressing a key enzyme, microbe expressing a key enzyme and yeast expressing a key enzyme.

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31. The method of claim 25, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

32. A method for producing fermentable sugar comprising:

a) providing transgenic plant material comprising one or more lock enzymes, one or more locked carbohydrates and one or more key enzymes; and

b) processing said transgenic plant material under conditions sufficient for said one or more key enzymes to convert said one or more locked carbohydrates to fermentable sugar.

33. The method of claim 32, wherein the one or more lock enzymes is selected from the group consisting of dextranucrase, levan sucrose, alternansucrase, sucrose isomerase and amylosucrase.

34. The method of claim 32, wherein the one or more key enzymes is targeted away from the one or more locked carbohydrates.

35. The method of claim 32, wherein the one or more key enzymes is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

36. The method of claim 32, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

37. The method of claim 32, wherein the one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

38. The method of claim 32, wherein the one or more key enzymes is provided by a source selected from the group consisting of transgenic plant material expressing a key enzyme, recombinant microbe expressing a key enzyme, transgenic yeast expressing a key enzyme, microbe expressing a key enzyme and yeast expressing a key enzyme.

39. The method of claim 32, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

40. A transgenic plant comprising one or more heterologous lock enzymes and one or more heterologous key enzymes.

41. The transgenic plant of claim 40, wherein the one or more lock enzymes is selected from the group consisting of dextranucrase, levan sucrose, alternansucrase, sucrose isomerase and amylosucrase.

42. The transgenic plant of claim 40, wherein the one or more key enzymes is targeted away from the locked carbohydrate.

43. The transgenic plant of claim 40, wherein the one or more key enzymes is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

44. The transgenic plant of claim 40, wherein the locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

45. The transgenic plant of claim 40, wherein the one or more key enzyme is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

46. The transgenic plant of claim 40, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

47. A transgenic plant comprising one or more locked carbohydrates and one or more key enzymes.

48. The transgenic plant of claim 47, wherein the one or more key enzymes is targeted away from the one or more locked carbohydrates.

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49. The transgenic plant of claim 47, wherein the key enzyme is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

50. The transgenic plant of claim 47, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltose, turanose and isomaltose.

51. The transgenic plant of claim 47, wherein the one or more key enzyme is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase.

52. The transgenic plant of claim 47, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

53. A method for producing fermentable sugar comprising:

a) providing transgenic plant material wherein said transgenic plant material is selected from the group consisting of sugar beet, sorghum, maize, and sugarcane, and wherein said transgenic plant material comprises:

i) one or more lock enzymes wherein said one or more lock enzymes is selected from the group consisting of dextranase, levan sucrose, alternansucrase, sucrose isomerase and amylosucrase,

ii) one or more locked carbohydrates wherein said one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextrans, fructans, maltose, turanose and isomaltose,

iii) one or more key enzymes wherein said one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase; and wherein said one or more key enzymes is targeted away from said one or more locked carbohydrates; and

b) processing said transgenic plant material under conditions sufficient for said one or more key enzymes to convert said one or more locked carbohydrates to fermentable sugar.

54. A transgenic plant comprising:

a) one or more lock enzymes wherein said one or more lock enzymes is selected from the group consisting of dextranase, levan sucrose, alternansucrase, sucrose isomerase and amylosucrase,

b) one or more locked carbohydrates wherein said one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextrans, fructans, maltose, turanose and isomaltose,

c) one or more key enzymes wherein said one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, alpha-1,5-glucosidase, alpha-1,1-glucosidase and alpha-1,6-glucosidase; and wherein said one or more key enzymes is targeted away from the one or more locked carbohydrates, and

d) wherein said transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

55. A method for producing fermentable sugar derived from a plant comprising:

a) providing plant material comprising locked carbohydrate; and,

b) contacting said plant material with one or more enzymes capable of converting the locked carbohydrate into fermentable sugar (key enzyme), wherein said contacting is under conditions sufficient for said conversion.

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56. The method of embodiment 55, wherein said plant material comprising locked carbohydrate is derived from a transgenic plant expressing one or more enzymes capable of converting an endogenous carbohydrate of said transgenic plant into said locked carbohydrate (lock enzyme).

57. The method of embodiment 55 or 56, wherein the key enzyme is provided as a purified or semi-purified enzyme preparation.

58. The method of embodiment 55 or 56, wherein at least one of the key enzymes is provided as plant material derived from a plant expressing said key enzyme.

59. The method of embodiment 58, wherein at least one of the key enzymes is expressed in the same plant as the plant comprising the locked carbohydrate.

60. The method of embodiment 55, wherein the locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, dextran, fructan, amylose, leucrose and alternan.

61. The method of embodiment 56, wherein the transgenic plant expresses at least two sucrose isomerase enzymes, wherein at least the first sucrose isomerase enzyme catalyzes the conversion of sucrose primarily into isomaltulose, and wherein at least the second sucrose isomerase enzyme catalyzes the conversion of sucrose primarily into trehalulose.

62. The method of embodiment 55, wherein said plant material comprising the locked carbohydrate is derived from a plant selected from the group consisting of maize, wheat, rice, barley, soybean, cotton, sorghum, oats, tobacco, *Miscanthus* grass, Switch grass, trees, beans, rape/canola, alfalfa, flax, sunflower, safflower, millet, rye, sugarcane, sugar beet, cocoa, tea, *Brassica*, cotton, coffee, sweet potato, flax, peanut, clover; vegetables such as lettuce, tomato, cucurbits, cassava, potato, carrot, radish, pea, lentils, cabbage, cauliflower, broccoli, Brussels sprouts, peppers, and pineapple; tree fruits such as citrus, apples, pears, peaches, apricots, walnuts, avocado, banana, and coconut; and flowers such as orchids, carnations and roses.

63. The method of embodiment 62, wherein said plant material comprising the locked carbohydrate is derived from sugarcane, sugar beet, or sweet sorghum.

64. The method of embodiment 55, wherein the key enzyme is derived from a microorganism.

65. The method of embodiment 64, wherein the key enzyme is endogenous to said microorganism.

66. The method of embodiment 64, wherein the key enzyme is a recombinant enzyme expressed in the microorganism.

67. The method of embodiment 65, wherein the microorganism is a *Saccharomyces* strain capable of fermenting isomaltulose.

68. A method of selecting a transformed plant comprising:

a) introducing into said plant or part thereof:

i) an expression cassette comprising a nucleotide sequence encoding an enzyme capable of converting an endogenous sugar in said plant to a locked carbohydrate; and,

ii) an expression cassette comprising a nucleotide sequence encoding an enzyme capable of converting the locked carbohydrate into a fermentable sugar;

b) maintaining said plant or part thereof under conditions sufficient for the expression of the lock enzyme and the key enzyme; and,

c) evaluating the sugar profile of said plant;

wherein the presence of one or more of the fermentable sugars produced by said key enzyme is indicative of a transformed plant.

69. A transgenic plant useful for the production of ethanol, wherein said plant comprises:

- a) a nucleotide sequence encoding an enzyme capable of converting an endogenous sugar in said plant to said locked carbohydrate; and,
- b) a nucleotide sequence encoding an enzyme capable of converting the locked carbohydrate into a fermentable sugar.

70. The plant of embodiment 69, wherein the locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, dextran, fructan, amylose, leucrose and alternan.

71. The plant of embodiment 70, wherein the transgenic plant expresses at least two sucrose isomerase enzymes, wherein at least the first sucrose isomerase enzyme catalyzes the conversion of sucrose primarily into isomaltulose, and wherein at least the second sucrose isomerase enzyme catalyzes the conversion of sucrose primarily into trehalulose.

72. The transgenic plant of embodiment 69 selected from the group consisting of maize, wheat, rice, barley, soybean, cotton, sorghum, oats, tobacco, *Miscanthus* grass, Switch grass, trees, beans, rape/canola, alfalfa, flax, sunflower, safflower,

millet, rye, sugarcane, sugar beet, cocoa, tea, *Brassica*, cotton, coffee, sweet potato, flax, peanut, clover; vegetables such as lettuce, tomato, cucurbits, cassava, potato, carrot, radish, pea, lentils, cabbage, cauliflower, broccoli, Brussels sprouts, peppers, and pineapple; tree fruits such as citrus, apples, pears, peaches, apricots, walnuts, avocado, banana, and coconut; and flowers such as orchids, carnations and roses.

73. The plant of embodiment 62, wherein said plant is sugarcane, sugar beet, or sorghum.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 58

<210> SEQ ID NO 1
 <211> LENGTH: 562
 <212> TYPE: PRT
 <213> ORGANISM: (Geo)Bacillus thermoglucosidasius KP1006
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(562)
 <223> OTHER INFORMATION: alpha-1,6-glucosidase

<400> SEQUENCE: 1

```

Met Glu Arg Val Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro
1          5          10          15

Arg Ser Phe Tyr Asp Ser Asn Gly Asp Gly Ile Gly Asp Ile Arg Gly
          20          25          30

Ile Ile Ala Lys Leu Asp Tyr Leu Lys Glu Leu Gly Val Asp Val Val
          35          40          45

Trp Leu Ser Pro Val Tyr Lys Ser Pro Asn Asp Asp Asn Gly Tyr Asp
          50          55          60

Ile Ser Asp Tyr Arg Asp Ile Met Asp Glu Phe Gly Thr Met Ala Asp
          65          70          75          80

Trp Lys Thr Met Leu Glu Glu Met His Lys Arg Gly Ile Lys Leu Val
          85          90          95

Met Asp Leu Val Val Asn His Thr Ser Asp Glu His Pro Trp Phe Ile
          100          105          110

Glu Ser Arg Lys Ser Lys Asp Asn Pro Tyr Arg Asp Tyr Tyr Ile Trp
          115          120          125

Arg Pro Gly Lys Asn Gly Lys Glu Pro Asn Asn Trp Glu Ser Val Phe
          130          135          140

Ser Gly Ser Ala Trp Glu Tyr Asp Glu Met Thr Gly Glu Tyr Tyr Leu
          145          150          155          160

His Leu Phe Ser Lys Lys Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys
          165          170          175

Val Arg Arg Glu Val Tyr Glu Met Met Lys Phe Trp Leu Asp Lys Gly
          180          185          190

Val Asp Gly Phe Arg Met Asp Val Ile Asn Met Ile Ser Lys Val Pro
          195          200          205

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-continued

Glu Leu Pro Asp Gly Glu Pro Gln Ser Gly Lys Lys Tyr Ala Ser Gly
 210 215 220
 Ser Arg Tyr Tyr Met Asn Gly Pro Arg Val His Glu Phe Leu Gln Glu
 225 230 235 240
 Met Asn Arg Glu Val Leu Ser Lys Tyr Asp Ile Met Thr Val Gly Glu
 245 250 255
 Thr Pro Gly Val Thr Pro Lys Glu Gly Ile Leu Tyr Thr Asp Pro Ser
 260 265 270
 Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Leu Asp
 275 280 285
 Ser Gly Pro Gly Gly Lys Trp Asp Ile Arg Pro Trp Ser Leu Ala Asp
 290 295 300
 Leu Lys Lys Thr Met Thr Lys Trp Gln Lys Glu Leu Glu Gly Lys Gly
 305 310 315 320
 Trp Asn Ser Leu Tyr Leu Asn Asn His Asp Gln Pro Arg Ala Val Ser
 325 330 335
 Arg Phe Gly Asp Asp Gly Lys Tyr Arg Val Glu Ser Ala Lys Met Leu
 340 345 350
 Ala Thr Phe Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly
 355 360 365
 Glu Glu Ile Gly Met Thr Asn Val Arg Phe Pro Ser Ile Glu Asp Tyr
 370 375 380
 Arg Asp Ile Glu Thr Leu Asn Met Tyr Lys Glu Arg Val Glu Glu Tyr
 385 390 395 400
 Gly Glu Asp Pro Gln Glu Val Met Glu Lys Ile Tyr Tyr Lys Gly Arg
 405 410 415
 Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Glu Asn Ala Gly
 420 425 430
 Phe Thr Ala Gly Thr Pro Trp Ile Pro Val Asn Pro Asn Tyr Lys Glu
 435 440 445
 Ile Asn Val Lys Ala Ala Leu Glu Asp Pro Asn Ser Val Phe His Tyr
 450 455 460
 Tyr Lys Lys Leu Ile Gln Leu Arg Lys Gln His Asp Ile Ile Val Tyr
 465 470 475 480
 Gly Thr Tyr Asp Leu Ile Leu Glu Asp Asp Pro Tyr Ile Tyr Arg Tyr
 485 490 495
 Thr Arg Thr Leu Gly Asn Glu Gln Leu Ile Val Ile Thr Asn Phe Ser
 500 505 510
 Glu Lys Thr Pro Val Phe Arg Leu Pro Asp His Ile Ile Tyr Lys Thr
 515 520 525
 Lys Glu Leu Leu Ile Ser Asn Tyr Asp Val Asp Glu Ala Glu Glu Leu
 530 535 540
 Lys Glu Ile Arg Leu Arg Pro Trp Glu Ala Arg Val Tyr Lys Ile Arg
 545 550 555 560
 Leu Pro

<210> SEQ ID NO 2

<211> LENGTH: 551

<212> TYPE: PRT

<213> ORGANISM: *Erwinia rhapontici* DSM 4484

<220> FEATURE:

<221> NAME/KEY: enzyme

<222> LOCATION: (1)..(551)

<223> OTHER INFORMATION: alpha-1,6-glucosidase Genebank AAK28737

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<400> SEQUENCE: 2

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Met Arg Ser Thr Pro His Trp Lys Glu Ala Val Val Tyr Gln Val Tyr
1          5          10          15

Pro Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Thr Gly Asp Leu Asn
20          25          30

Gly Ile Ile Ser Lys Leu Asp Tyr Leu Gln Gln Leu Gly Ile Thr Leu
35          40          45

Leu Trp Leu Ser Pro Val Tyr Arg Ser Pro Met Asp Asp Asn Gly Tyr
50          55          60

Asp Ile Ser Asp Tyr Glu Glu Ile Ala Asp Ile Phe Gly Ser Met Ser
65          70          75          80

Asp Met Glu Arg Leu Ile Ala Glu Ala Lys Ala Arg Asp Ile Gly Ile
85          90          95

Leu Met Asp Leu Val Val Asn His Thr Ser Asp Glu His Pro Trp Phe
100         105         110

Ile Asp Ala Leu Ser Ser Lys Asn Ser Ala Tyr Arg Asp Phe Tyr Ile
115         120         125

Trp Arg Ala Pro Ala Ala Asp Gly Gly Pro Pro Asp Asp Ser Arg Ser
130         135         140

Asn Phe Gly Gly Ser Ala Trp Thr Leu Asp Glu Ala Ser Gly Glu Tyr
145         150         155         160

Tyr Leu His Gln Phe Ser Thr Arg Gln Pro Asp Leu Asn Trp Glu Asn
165         170         175

Pro Arg Val Arg Glu Ala Ile His Ala Met Met Asn Arg Trp Leu Asp
180         185         190

Lys Gly Ile Gly Gly Phe Arg Met Asp Val Ile Asp Leu Ile Gly Lys
195         200         205

Glu Val Asp Pro Gln Ile Met Ala Asn Gly Arg His Pro His Leu Tyr
210         215         220

Leu Gln Gln Met Asn Arg Ala Thr Phe Gly Pro Arg Gly Ser Val Thr
225         230         235         240

Val Gly Glu Thr Trp Ser Ala Thr Pro Glu Asp Ala Leu Leu Tyr Ser
245         250         255

Ala Glu Glu Arg Gln Glu Arg Gln Glu Leu Thr Met Val Phe Gln Phe
260         265         270

Glu His Ile Lys Leu Phe Trp Asp Glu Gln Tyr Gly Lys Trp Cys Asn
275         280         285

Gln Pro Phe Asp Leu Leu Arg Phe Lys Ala Val Ile Asp Lys Trp Gln
290         295         300

Thr Ala Leu Ala Asp His Gly Trp Asn Ser Leu Phe Trp Ser Asn His
305         310         315         320

Asp Leu Pro Arg Ala Val Ser Lys Phe Gly Asp Asp Gly Glu Tyr Arg
325         330         335

Val Val Ser Ala Lys Met Leu Ala Thr Ala Leu His Cys Leu Lys Gly
340         345         350

Thr Pro Tyr Ile Tyr Gln Gly Glu Glu Ile Gly Met Thr Asn Val Asn
355         360         365

Phe Ala Asp Ile Asp Asp Tyr Arg Asp Ile Glu Ser Leu Asn Leu Tyr
370         375         380

Gln Glu Arg Ile Ala Glu Gly Met Ser His Glu Ala Met Met Arg Gly
385         390         395         400

Ile His Ala Asn Gly Pro Asp Asn Ala Arg Thr Pro Met Gln Trp Thr
405         410         415

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Ala Val His Met Pro Gly Leu Pro Pro Val Ser Pro Gly Leu Arg Leu
420 425 430

Ile Leu Thr Ser Gly Gln Trp Asn Val Ala Ala Ala Leu Asp Asp Pro
435 440 445

Asp Ser Val Phe Tyr His Tyr Gln Lys Leu Val Ala Leu Arg Lys Gln
450 455 460

Leu Pro Leu Leu Val His Gly Asp Phe Arg Gln Ile Val Val Glu His
465 470 475 480

Pro Gln Val Phe Ala Trp Leu Arg Thr Leu Gly Glu Gln Thr Leu Val
485 490 495

Val Ile Asn Asn Phe Thr Arg Asp Ala Val Met Leu Ala Ile Pro Asp
500 505 510

Asn Leu Gln Ser Gln Gln Gly Arg Cys Leu Ile Asn Asn Tyr Ala Pro
515 520 525

Arg Glu Gln Leu Glu Pro Ile Met Glu Leu Gln Pro Tyr Glu Ser Phe
530 535 540

Ala Leu Leu Ile Glu Arg Leu
545 550

<210> SEQ ID NO 3
<211> LENGTH: 564
<212> TYPE: PRT
<213> ORGANISM: Bacillus thuringiensis str. Al Hakam
<220> FEATURE:
<221> NAME/KEY: enzyme
<222> LOCATION: (1)..(564)
<223> OTHER INFORMATION: alpha-1,6-glucosidase

<400> SEQUENCE: 3

Met Lys Trp Gly Ser Ile Met Glu Lys Gln Trp Trp Lys Glu Ser Val
1 5 10 15

Val Tyr Gln Ile Tyr Pro Arg Ser Phe Met Asp Ser Asn Gly Asp Gly
20 25 30

Ile Gly Asp Leu Arg Gly Ile Ile Ser Lys Leu Asp Tyr Leu Lys Glu
35 40 45

Leu Gly Ile Asp Val Ile Trp Leu Ser Pro Val Tyr Glu Ser Pro Asn
50 55 60

Asp Asp Asn Gly Tyr Asp Ile Ser Asp Tyr Cys Lys Ile Met Asn Glu
65 70 75 80

Phe Gly Thr Met Glu Asp Trp Asp Glu Leu Leu His Glu Met His Glu
85 90 95

Arg Asn Met Lys Leu Met Met Asp Leu Val Val Asn His Thr Ser Asp
100 105 110

Glu His Asn Trp Phe Ile Glu Ser Arg Lys Ser Lys Asp Asn Lys Tyr
115 120 125

Arg Asp Tyr Tyr Ile Trp Arg Pro Gly Lys Glu Gly Lys Glu Pro Asn
130 135 140

Asn Trp Gly Ala Ala Phe Ser Gly Ser Ala Trp Gln Tyr Asp Glu Met
145 150 155 160

Thr Asp Glu Tyr Tyr Leu His Leu Phe Ser Lys Lys Gln Pro Asp Leu
165 170 175

Asn Trp Asp Asn Glu Lys Val Arg Gln Asp Val Tyr Glu Met Met Lys
180 185 190

Phe Trp Leu Glu Lys Gly Ile Asp Gly Phe Arg Met Asp Val Ile Asn
195 200 205

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Phe Ile Ser Lys Glu Glu Gly Leu Pro Thr Val Glu Thr Glu Glu Glu
 210                215                220

Gly Tyr Val Ser Gly His Lys His Phe Met Asn Gly Pro Asn Ile His
 225                230                235                240

Lys Tyr Leu His Glu Met Asn Glu Glu Val Leu Ser His Tyr Asp Ile
                245                250                255

Met Thr Val Gly Glu Met Pro Gly Val Thr Thr Glu Glu Ala Lys Leu
                260                265                270

Tyr Thr Gly Glu Glu Arg Lys Glu Leu Gln Met Val Phe Gln Phe Glu
                275                280                285

His Met Asp Leu Asp Ser Gly Glu Gly Gly Lys Trp Asp Val Lys Pro
 290                295                300

Cys Ser Leu Leu Thr Leu Lys Glu Asn Leu Thr Lys Trp Gln Lys Ala
 305                310                315                320

Leu Glu His Thr Gly Trp Asn Ser Leu Tyr Trp Asn Asn His Asp Gln
                325                330                335

Pro Arg Val Val Ser Arg Phe Gly Asn Asp Gly Met Tyr Arg Ile Glu
                340                345                350

Ser Ala Lys Met Leu Ala Thr Val Leu His Met Met Lys Gly Thr Pro
                355                360                365

Tyr Ile Tyr Gln Gly Glu Glu Ile Gly Met Thr Asn Val Arg Phe Glu
 370                375                380

Ser Ile Asp Glu Tyr Arg Asp Ile Glu Thr Leu Asn Met Tyr Lys Glu
 385                390                395                400

Lys Val Met Glu Arg Gly Glu Asp Ile Glu Lys Val Met Gln Ser Ile
                405                410                415

Tyr Ile Lys Gly Arg Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp
                420                425                430

Gln Asn His Ala Gly Phe Thr Thr Gly Glu Pro Trp Ile Thr Val Asn
                435                440                445

Pro Asn Tyr Lys Glu Ile Asn Val Lys Gln Ala Ile Gln Asn Lys Asp
 450                455                460

Ser Ile Phe Tyr Tyr Tyr Lys Lys Leu Ile Glu Leu Arg Lys Asn Asn
 465                470                475                480

Glu Ile Val Val Tyr Gly Ser Tyr Asp Leu Ile Leu Glu Asn Asn Pro
                485                490                495

Ser Ile Phe Ala Tyr Val Arg Thr Tyr Gly Val Glu Lys Leu Leu Val
                500                505                510

Ile Ala Asn Phe Thr Ala Glu Glu Cys Ile Phe Glu Leu Pro Glu Asp
                515                520                525

Ile Ser Tyr Ser Glu Val Glu Leu Leu Ile His Asn Tyr Asp Val Glu
 530                535                540

Asn Gly Pro Ile Glu Asn Ile Thr Leu Arg Pro Tyr Glu Ala Met Val
 545                550                555                560

Phe Lys Leu Lys

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<210> SEQ ID NO 4
<211> LENGTH: 576
<212> TYPE: PRT
<213> ORGANISM: Arthrobacter globiformis
<220> FEATURE:
<221> NAME/KEY: enzyme
<222> LOCATION: (1)..(576)
<223> OTHER INFORMATION: alpha-1,6-glucosidase Genebank AB113246

<400> SEQUENCE: 4

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Met 1	Thr	Ile	Glu	Glu 5	Thr	Glu	Glu	Glu	Ala 10	Thr	Tyr	Arg	Ala	Gly 15	Arg
Glu	Trp	Phe	Lys 20	Ser	Ala	Val	Val	Tyr 25	Gln	Ile	Tyr	Pro	Arg 30	Ser	Phe
Ala	Asp	Ser	Asp	Gly	Asp	Gly	Val 40	Gly	Asp	Leu	Arg	Gly 45	Ile	Ile	Gly
Lys	Leu	Asp	Tyr	Leu	Gln	Lys 55	Leu	Gly	Val	Asp	Val 60	Val	Trp	Leu	Ser
Pro 65	Val	Tyr	Arg	Ser	Pro	Gln	Asp	Asp	Asn	Gly 75	Tyr	Asp	Ile	Ser	Asp 80
Tyr	Arg	Glu	Ile	Asp 85	Pro	Val	Phe	Gly	Gly 90	Leu	Glu	Thr	Leu 95	Asp	Glu
Leu	Leu	Asp	Gly 100	Leu	His	Ala	Arg	Gly 105	Met	Lys	Leu	Val	Met 110	Asp	Leu
Val	Val	Asn	His	Thr	Ser	Asp	Glu 120	His	Pro	Trp	Phe	Val	Glu 125	Ser	Arg
Ser	Ser	Lys	Asp	Ser	Pro	Lys 135	Arg	Asp	Trp	Tyr	Trp	Trp	Arg	Pro	Ala
Arg 145	Glu	Gly	Ala	Glu	Pro	Gly	Thr	Ala	Gly	Ala 155	Glu	Pro	Asn	Asn	Trp 160
Gly	Ser	Ala	Phe	Ser 165	Gly	Pro	Ala	Trp	Glu	Tyr	Asp	Ala	Ala	Thr	Gly 175
Glu	Tyr	Tyr	Leu 180	His	Leu	Phe	Ser	Arg	Lys	Gln	Pro	Asp	Leu 190	Asn	Trp
Glu	Asn	Pro	Glu	Val	Arg	Ala	Ala 200	Val	Tyr	Asp	Met	Met 205	Asn	Trp	Trp
Leu	Asp	Arg	Gly 210	Val	Asp	Gly	Phe	Arg	Met	Asp	Val 220	Ile	Asn	Phe	Ile
Ser 225	Lys	Asp	Gln	Thr	Leu	Pro	Asp	Gly	Pro	Arg 235	Ala	Asp	Gly	Met	Leu 240
Phe	Gly	Asp	Gly	Gly 245	Pro	His	Tyr	Ile	Cys	Gly 250	Pro	Arg	Ile	His	Glu 255
Phe	Leu	Gln	Glu	Met	His	Gln	Glu	Val 265	Phe	Ala	Gly	Arg	Asp 270	Lys	Asp
Leu	Leu	Thr	Val	Gly	Glu	Met	Pro 280	Gly	Val	Thr	Val	Asp 285	Glu	Ala	Val
Leu	Phe	Thr	Asp	Pro	Gly	Arg 295	Arg	Glu	Val	Asp	Met 300	Val	Phe	Gln	Phe
Glu 305	His	Val	Ala	Leu	Asp 310	Gln	Glu	Gly	Gly	Asn 315	Lys	Trp	Arg	Pro	Lys 320
Lys	Leu	Leu	Leu	Thr 325	Asp	Leu	Lys	Lys	Ser	Leu	Gly	Arg	Trp	Gln	Glu 335
Ala	Leu	Gly	Glu	Arg	Gly	Trp	Asn	Ser 345	Leu	Tyr	Trp	Gly	Asn 350	His	Asp
Gln	Ala	Arg	Ala	Val	Ser	Arg	Phe 360	Gly	Asp	Asp	Gly	Glu 365	Tyr	Arg	Glu
Gln 370	Ser	Ala	Lys	Met	Leu	Ala	Ala 375	Val	Leu	His	Leu	His 380	Arg	Gly	Thr
Pro 385	Tyr	Val	Tyr	Gln	Gly 390	Glu	Glu	Leu	Gly	Met 395	Thr	Asn	Met	Ala	Phe 400
Gly	Ala	Ile	Ser	Asp 405	Tyr	Arg	Asp	Ile	Glu	Val	Leu	Asn	His	His	Arg 415

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Glu Ala Thr Thr His Leu Gly His Thr Asp Ala Glu Val Leu Ala Ala
 420 425 430
 Leu Ala Pro Leu Asn Arg Asp Asn Ala Arg Thr Pro Val Gln Trp Asp
 435 440 445
 Ala Ser Arg His Gly Gly Phe Thr Thr Gly Ala Pro Trp Ile Ala Val
 450 455 460
 Asn Pro Asn Ala Asn Thr Ile Asn Ala Ala Ala Gln Val Asp Asp Pro
 465 470 475 480
 Asp Ser Val Phe Ser Phe Tyr Arg Arg Val Ile Ala Leu Arg His Ala
 485 490 495
 Asp Pro Val Val Ala Tyr Gly Asp Phe Thr Met Leu Leu Pro Asp Asp
 500 505 510
 Glu His Val Tyr Ala Phe Arg Arg Ser Leu Pro Asp Ala Glu Leu Leu
 515 520 525
 Val Leu Gly Asn Phe Ser Gly Thr Gly Gln Ser Ala Gly Val Asp Gly
 530 535 540
 Ser Trp Gly Asp Ala Glu Leu Val Leu Gly Asn Tyr Pro Ala Ala Pro
 545 550 555 560
 Gly Leu Gly Leu Arg Pro Trp Glu Val Lys Val Phe Arg Arg Asn Leu
 565 570 575

<210> SEQ ID NO 5
 <211> LENGTH: 787
 <212> TYPE: PRT
 <213> ORGANISM: Bacillus thermoamyloliquefaciens
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(787)
 <223> OTHER INFORMATION: alpha-1,6-glucosidase Q9F234

<400> SEQUENCE: 5

Met Leu Glu Asp Thr Ser Phe Ala Ile Gln Pro Glu Gln Asp Asp Lys
 1 5 10 15
 Thr Gln Glu Thr His Arg Ile Asp Ile Gly Asn Met His Thr Phe Ser
 20 25 30
 His Thr Glu His Val Phe Ser Phe His Cys Asp Thr Gly Ile Val Lys
 35 40 45
 Ile Arg Phe Tyr Arg Glu Asp Ile Val Arg Ile Ala Phe Asn Pro Phe
 50 55 60
 Gly Glu Thr Ser Leu Ser Thr Ser Val Ala Val Val Lys Glu Pro Glu
 65 70 75 80
 Lys Val Asp Ala Ser Val His Glu Thr Glu Glu Glu Val Thr Leu Thr
 85 90 95
 Ser Ala Lys Gln Thr Val Val Leu Gln Lys Arg Pro Phe Arg Val Arg
 100 105 110
 Ile Tyr Asp Asn His Gly Arg Leu Leu Val Ala Glu Gly Lys Lys Gly
 115 120 125
 Met Ala Phe Thr Tyr Gln Gly Glu Val Cys Cys Phe Lys Met Met Asp
 130 135 140
 Glu Ala Asp His Phe Tyr Gly Phe Gly Glu Lys Thr Gly Phe Leu Asp
 145 150 155 160
 Lys Arg Gly Glu Thr Met Thr Met Trp Asn Thr Asp Val Tyr Ala Pro
 165 170 175
 His Asn Pro Glu Thr Asp Pro Leu Tyr Gln Ser His Pro Tyr Phe Met
 180 185 190
 Thr Val Arg Asn Gly Ser Ala His Gly Ile Phe Phe Asp Asn Thr Tyr

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195					200					205					
Lys	Thr	Thr	Phe	Asp	Phe	Gln	Thr	Ala	Thr	Asp	Glu	Tyr	Cys	Phe	Ser
210					215					220					
Ala	Glu	Gly	Gly	Ala	Ile	Asp	Tyr	Tyr	Val	Phe	Ala	Gly	Pro	Thr	Pro
225					230					235					240
Lys	Asp	Val	Leu	Glu	Gln	Tyr	Thr	Asp	Leu	Thr	Gly	Arg	Met	Pro	Leu
			245						250					255	
Pro	Pro	Lys	Trp	Ala	Leu	Gly	Tyr	His	Gln	Ser	Arg	Tyr	Ser	Tyr	Glu
		260						265					270		
Thr	Glu	Gln	Glu	Val	Arg	Glu	Ile	Ala	Gln	Thr	Phe	Ile	Glu	Lys	Asp
	275						280					285			
Ile	Pro	Leu	Asp	Val	Ile	Tyr	Leu	Asp	Ile	His	Tyr	Met	Asn	Gly	Tyr
290						295					300				
Arg	Val	Phe	Thr	Phe	Asp	Arg	Asn	Arg	Phe	Pro	Asn	Leu	Lys	Gln	Leu
305					310					315					320
Ile	Ala	Asp	Leu	Lys	Gln	Lys	Gly	Ile	Arg	Val	Val	Pro	Ile	Val	Asp
			325					330						335	
Pro	Gly	Val	Lys	Glu	Asp	Pro	Glu	Tyr	Val	Ile	Tyr	Gln	Glu	Gly	Ile
		340						345					350		
Arg	His	Asp	Tyr	Phe	Cys	Lys	Tyr	Ile	Glu	Gly	Asn	Val	Tyr	Phe	Gly
		355					360					365			
Glu	Val	Trp	Pro	Gly	Lys	Ser	Ala	Phe	Pro	Asp	Phe	Thr	Asn	Lys	Lys
370						375					380				
Val	Arg	Lys	Trp	Trp	Gly	Glu	Lys	His	Gln	Phe	Tyr	Thr	Asp	Leu	Gly
385					390					395					400
Ile	Glu	Gly	Ile	Trp	Asn	Asp	Met	Asn	Glu	Pro	Ser	Val	Phe	Asn	Glu
			405						410					415	
Thr	Lys	Thr	Met	Asp	Val	Lys	Val	Ile	His	Asp	Asn	Asp	Gly	Asp	Pro
		420						425					430		
Lys	Thr	His	Arg	Glu	Leu	His	Asn	Val	Tyr	Gly	Phe	Met	Met	Gly	Glu
		435					440					445			
Ala	Thr	Tyr	Lys	Gly	Met	Lys	Lys	Leu	Leu	Asn	Gly	Lys	Arg	Pro	Phe
450						455					460				
Leu	Leu	Thr	Arg	Ala	Gly	Phe	Ser	Gly	Ile	Gln	Arg	Tyr	Ala	Ala	Val
465					470					475					480
Trp	Thr	Gly	Asp	Asn	Arg	Ser	Phe	Trp	Glu	His	Leu	Gln	Met	Ser	Leu
			485						490					495	
Pro	Met	Cys	Met	Asn	Leu	Gly	Leu	Ser	Gly	Val	Ala	Phe	Cys	Gly	Pro
		500						505					510		
Asp	Val	Gly	Gly	Phe	Ala	His	Asn	Thr	Asn	Gly	Glu	Leu	Leu	Thr	Arg
		515					520					525			
Trp	Met	Gln	Val	Gly	Ala	Phe	Thr	Pro	Tyr	Phe	Arg	Asn	His	Cys	Ala
530						535					540				
Ile	Gly	Phe	Arg	Arg	Gln	Glu	Pro	Trp	Ala	Phe	Gly	Glu	Lys	Tyr	Glu
545					550					555					560
Arg	Ile	Ile	Lys	Lys	Tyr	Ile	Arg	Leu	Arg	Tyr	Gln	Trp	Leu	Pro	His
			565					570						575	
Leu	Tyr	Thr	Leu	Phe	Ala	Glu	Ala	His	Glu	Thr	Gly	Ala	Pro	Val	Met
		580						585					590		
Arg	Pro	Leu	Phe	Phe	Glu	Tyr	Pro	Asp	Asp	Glu	Asn	Thr	Tyr	Asn	Leu
		595					600					605			
Tyr	Asp	Glu	Phe	Leu	Val	Gly	Ala	Asn	Val	Leu	Ile	Ala	Pro	Ile	Met
610						615					620				

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Thr Pro Ser Thr Thr Arg Arg Val Ala Tyr Phe Pro Lys Gly Asn Trp
 625 630 635 640
 Val Asp Tyr Trp Thr Gly Glu Val Leu Glu Gly Gly Gln Tyr His Leu
 645 650 655
 Ile Ser Ala Asp Leu Glu Thr Leu Pro Ile Phe Ile Lys Gln Gly Ser
 660 665 670
 Ala Ile Ala Leu Gly Asp Val Lys Arg Ser Thr Glu Met Pro Asp Glu
 675 680 685
 His Arg Thr Val His Ile Tyr Lys Ala Asn Gly Gly Lys Ala Thr Tyr
 690 695 700
 Val Leu Tyr Asp Asp Asp Gly Gln Thr Phe Ser Tyr Glu Lys Gly Asp
 705 710 715 720
 Tyr Leu Arg Met Tyr Ile Glu Val Glu Tyr Gly Glu Asn Ser Val His
 725 730 735
 Ile Val Thr Lys Ser Glu Gly Thr Tyr Gln Pro Ser Trp Lys Leu Ser
 740 745 750
 Phe Ala Ile His His Ala Thr Glu Gln Thr Lys Val Thr Ile Asp Gly
 755 760 765
 Asn Glu Gln Asn Ala Ile Phe Asp Pro His Gln Arg Ile Leu Leu Ile
 770 775 780
 Gln Ser Glu
 785

<210> SEQ ID NO 6
 <211> LENGTH: 752
 <212> TYPE: PRT
 <213> ORGANISM: Thermoanaerobacter ethanolicus
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(752)
 <223> OTHER INFORMATION: alpha-1,6-glucosidase ABR26230

<400> SEQUENCE: 6

Met Tyr Gln Lys Thr Ser Glu Lys Ile Val Val Arg Asn Glu Gly Lys
 1 5 10 15
 Lys Leu Glu Leu Arg Val Leu Gly Asp Lys Ile Ile Asn Val Phe Val
 20 25 30
 Ser Asn Lys Glu Glu Lys Arg Lys Asp Thr Ile Ala Ile Glu Arg Lys
 35 40 45
 Glu Tyr Asp Thr Pro Glu Phe Ser Ile Ser Asp Glu Leu Glu Ser Ile
 50 55 60
 Leu Ile Glu Thr Asn Ser Leu Lys Val Lys Ile Asn Lys Asn Asp Leu
 65 70 75 80
 Ser Val Ser Phe Leu Asp Lys Asn Gly Asn Ile Ile Asn Glu Asp Tyr
 85 90 95
 Asn Gly Gly Ala Lys Phe Asn Glu Thr Asp Val Arg Cys Tyr Lys Lys
 100 105 110
 Leu Arg Glu Asp His Phe Tyr Gly Phe Gly Glu Lys Ala Gly Tyr Leu
 115 120 125
 Asp Lys Lys Gly Glu Arg Leu Glu Met Trp Asn Thr Asp Glu Phe Met
 130 135 140
 Thr His Asn Gln Thr Thr Lys Leu Leu Tyr Glu Ser Tyr Pro Phe Phe
 145 150 155 160
 Ile Gly Met Asn Asp Tyr His Thr Tyr Gly Ile Phe Leu Asp Asn Ser
 165 170 175

-continued

Phe	Arg	Ser	Phe	Phe	Asp	Met	Gly	Gln	Glu	Ser	Gln	Glu	Tyr	Tyr	Phe
			180					185					190		
Phe	Gly	Ala	Tyr	Gly	Gly	Gln	Met	Asn	Tyr	Tyr	Phe	Ile	Tyr	Gly	Glu
		195					200					205			
Asp	Ile	Lys	Glu	Val	Val	Glu	Asn	Tyr	Thr	Tyr	Leu	Thr	Gly	Arg	Ile
	210					215					220				
Ser	Leu	Pro	Pro	Leu	Trp	Val	Leu	Gly	Asn	Gln	Gln	Ser	Arg	Tyr	Ser
225					230					235					240
Tyr	Thr	Pro	Gln	Glu	Arg	Val	Leu	Glu	Val	Ala	Lys	Thr	Phe	Arg	Glu
				245					250					255	
Lys	Asp	Ile	Pro	Cys	Asp	Val	Ile	Tyr	Leu	Asp	Ile	Asp	Tyr	Met	Glu
			260					265					270		
Gly	Tyr	Arg	Val	Phe	Thr	Trp	Asn	Lys	Glu	Thr	Phe	Lys	Asn	His	Lys
		275					280					285			
Glu	Met	Leu	Lys	Gln	Leu	Lys	Glu	Met	Gly	Phe	Lys	Val	Val	Thr	Ile
	290					295					300				
Val	Asp	Pro	Gly	Val	Lys	Arg	Asp	Tyr	Asp	Tyr	His	Val	Tyr	Arg	Glu
305					310					315					320
Gly	Ile	Glu	Lys	Gly	Tyr	Phe	Val	Lys	Asp	Lys	Tyr	Gly	Ile	Thr	Tyr
			325						330					335	
Val	Gly	Lys	Val	Trp	Pro	Gly	Glu	Ala	Cys	Phe	Pro	Asp	Phe	Leu	Gln
			340					345					350		
Glu	Glu	Val	Arg	Tyr	Trp	Trp	Gly	Glu	Lys	His	Arg	Glu	Phe	Ile	Asn
		355					360					365			
Asp	Gly	Ile	Asp	Gly	Ile	Trp	Asn	Asp	Met	Asn	Glu	Pro	Ala	Val	Phe
	370					375					380				
Glu	Thr	Pro	Thr	Lys	Thr	Met	Pro	Glu	Asp	Asn	Ile	His	Ile	Leu	Asp
385					390					395					400
Gly	Glu	Lys	Val	Leu	His	Lys	Glu	Ala	His	Asn	Val	Tyr	Ala	Asn	Tyr
			405						410					415	
Met	Ala	Met	Ala	Thr	Arg	Asp	Gly	Phe	Leu	Arg	Ile	Arg	Pro	Asn	Glu
			420					425					430		
Arg	Pro	Phe	Val	Leu	Thr	Arg	Ala	Ala	Phe	Ser	Gly	Ile	Gln	Arg	Tyr
		435					440					445			
Ala	Ala	Met	Trp	Thr	Gly	Asp	Asn	Arg	Ser	Leu	Tyr	Glu	His	Leu	Leu
	450					455					460				
Met	Met	Met	Pro	Met	Leu	Met	Asn	Ile	Gly	Leu	Ser	Gly	Gln	Pro	Phe
465					470					475					480
Val	Gly	Ala	Asp	Val	Gly	Gly	Phe	Glu	Gly	Asp	Cys	His	Glu	Glu	Leu
			485						490					495	
Phe	Ile	Arg	Trp	Ile	Glu	Ala	Ala	Val	Phe	Thr	Pro	Phe	Leu	Arg	Val
			500					505					510		
His	Ser	Ala	Ile	Gly	Thr	Lys	Asp	Gln	Glu	Pro	Trp	Ser	Phe	Gly	Lys
		515					520					525			
Arg	Ala	Glu	Asp	Ile	Ser	Arg	Lys	Tyr	Ile	Lys	Met	Arg	Tyr	Glu	Leu
	530					535					540				
Leu	Pro	Tyr	Leu	Tyr	Asp	Leu	Phe	Tyr	Ile	Ala	Ser	Gln	Lys	Gly	Tyr
545					550					555					560
Pro	Ile	Met	Arg	Pro	Leu	Val	Phe	Glu	Tyr	Gln	Lys	Asp	Glu	Asn	Thr
				565					570					575	
His	Lys	Ile	Tyr	Asp	Glu	Phe	Met	Phe	Gly	Glu	Gly	Leu	Leu	Val	Ala
			580					585					590		
Pro	Val	Tyr	Leu	Pro	Ser	Lys	Glu	Arg	Arg	Glu	Val	Tyr	Leu	Pro	Glu

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595	600	605
Gly Ile Trp Tyr Asp Tyr Trp Thr Gly Lys Gly Phe Lys Gly Lys Asn 610 615 620		
Tyr Tyr Leu Val Asp Ala Pro Ile Glu Val Ile Pro Leu Phe Val Lys 625 630 635 640		
Glu Gly Gly Ile Leu Leu Lys Gln Gln Pro Gln Ser Phe Ile Gly Glu 645 650 655		
Lys Lys Leu Glu Glu Leu Thr Val Glu Ile Tyr Lys Gly Lys Glu Gly 660 665 670		
His Tyr Leu His Tyr Glu Asp Asp Gly Lys Ser Phe Asp Tyr Thr Lys 675 680 685		
Gly Val Tyr Asn Leu Phe Asp Ile Ser Phe Cys Tyr Lys Glu Gly Arg 690 695 700		
Met Asp Ile Lys Phe Asp Lys Ile His Phe Gly Tyr Asp Lys Gly Val 705 710 715 720		
Lys Lys Tyr Lys Phe Ile Phe Lys Asn Phe Asp Asp Ile Lys Glu Ile 725 730 735		
Lys Ile Asn Gly Glu Lys Val Glu Lys Glu Ser Cys Glu Ile Glu Leu 740 745 750		

<210> SEQ ID NO 7
 <211> LENGTH: 1717
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis
 <220> FEATURE:
 <221> NAME/KEY: promoter
 <222> LOCATION: (1) .. (1717)
 <223> OTHER INFORMATION: UBQ promoter

<400> SEQUENCE: 7

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ggagccaagt ctcataaacg ccattgtgga agaaagtctt gagttggtgg taatgtaaca      60
gagtagtaag aacagagaag agagagagtg tgagatacat gaattgtcgg gcaacaaaaa      120
tcctgaacat cttatcttag caaagagaaa gagttccgag tctgtagcag aagagtgagg      180
agaaatttaa gctcttgga cttgtgaattg ttccgcctct tgaatacttc ttcaatcctc      240
atatattctt cttctatggt acctgaaaac cggcatttaa tctcgcgggt ttattccggg      300
tcaacatttt ttttgttttg agttattatc tgggcttaat aacgcaggcc tgaaataaat      360
tcaaggccca actgtttttt tttttaagaa gttgctgtta aaaaaaaaaa aagggaatta      420
acaacaacaa caaaaaaaga taaagaaaat aataacaatt actttaattg tagactaaaa      480
aaacatagat tttatcatga aaaaaagaga aaagaaataa aaacttggat caaaaaaaaaa      540
acatacagat cttctaatta ttaacttttc ttaaaaatta ggtccttttt cccaacaatt      600
aggttttagag ttttggaatt aaacaaaaaa gattgttcta aaaaatactc aaatttggtg      660
gataagtttc cttatcttaa ttagtcaatg gtagatactt tttttcttt tctttattag      720
agtagattag aatcttttat gccaaagtatt gataaattaa atcaagaaga taaactatca      780
taatcaacat gaaattaaaa gaaaaatctc atatatagta ttagtattct ctatatatat      840
tatgattgct tattcttaat gggttgggtt aaccaagaca tagtcttaat ggaaagaatc      900
ttttttgaac ttttctcta ttgattaaat tcttctatag aaaagaaaga aattatttga      960
ggaaaagtat atacaaaaag aaaaatagaa aaatgtcagt gaagcagatg taatggatga     1020
cctaatacaa ccaccaccaa aggatgtttc tacttgagtc ggtcttttaa aaacgcacgg     1080
tgaaaaatat gacacgtatc atatgattcc ttccttttagt ttcgtgataa taatcctcaa     1140

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ctgatatctt cctttttttg ttttggetaa agatatttta ttctcattaa tagaaaagac	1200
ggttttgggc ttttggtttg cgatataaag aagaccttcg tgtggaagat aataattcat	1260
cctttcgtct ttttctgact cttcaatctc tcccaaagcc taaagcgatc tctgcaaate	1320
tctcgcgact ctctctttca aggtatatatt tctgattctt tttgtttttg attcgatatct	1380
gatctccaat ttttgttatg tggattattg aatcttttgt ataaattgct tttgacaata	1440
ttgttcgttt cgtcaatoca gcttctaaat tttgtcctga ttactaagat atcgattcgt	1500
agtgtttaca tctgtgtaat ttcttgcttg attgtgaaat taggattttc aaggacgatc	1560
tattcaattt ttgtgttttc tttgttcgat tctctctggt ttaggtttct tatgtttaga	1620
tccgtttctc tttgggtgtg ttttgatttc tcttacggct tttgatttgg tatatgttcg	1680
ctgattgggt tctacttggt ctattgtttt atttcag	1717

<210> SEQ ID NO 8
 <211> LENGTH: 158
 <212> TYPE: DNA
 <213> ORGANISM: beet curly top virus
 <220> FEATURE:
 <221> NAME/KEY: rep_origin
 <222> LOCATION: (1)..(158)

<400> SEQUENCE: 8

attgaatcgg gctctcttca aatccctat caattgggtg ctttgggtgc tcttaaatac	60
caccaagggg ccattccgat taattattac ggatggcccc caaaaaatac gtggccaat	120
gaaaatatgc cactgggaaa gctaaagatg ttgatgtg	158

<210> SEQ ID NO 9
 <211> LENGTH: 1546
 <212> TYPE: DNA
 <213> ORGANISM: beet curly top virus
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(1546)
 <223> OTHER INFORMATION: BCTV replicase

<400> SEQUENCE: 9

tcaatatgcc tttttacaaa aaagccaaaa attttttcct tacataccct caatgttcag	60
taaccaaaga agacgcctta gaacagctcc tcgctataaa tacaccttcg aataaaaaat	120
atattcgcat ctgcagagaa ttacatgaaa atggggaacc acatctgcat gcccttattc	180
aattcgaagg aaaagtccag atccgtaatg cccgttactt cgatctgcaa catcgaagta	240
ccagcaaaca attccactgc aatattcagg gagctaaatc cagttccgac gtcaagtcct	300
acgtctcaaa ggacggagat cacatcgact ggggtgaatt tcaggctgat ggaagatctg	360
cacgcggagg tcaacagacg gctaattgat ctgcagcaga ggcattaaat gcaggtaatg	420
cattagaagc tctgcagata ataagggaga aactcccaga aaaatatatt ttccagtatc	480
acaacctcaa acctaactta gaggtatttt ttcttctctc tccagatctt tatcaaccac	540
cttttctctt ttcttcttct actagagttc cagaaattat tcaagagtgg gccgattctt	600
atttttggtt ggatcccgtc gcgcggcctt ttagatataa tagtttaate atagaggggtg	660
attcaagaac tggtaaaaaca atgtggggca ggtgtttagg tccacataat tatattactg	720
gtcatttaga ttttagttta aaaacttata atgataatgt tctgtataac gtcattgatg	780
acgtagatcc caattactta aagatgaagc attggaagca ccttataggc gcacaaagag	840
agtggcagac aaacttaaag tatggaaaac cactgtcat taaagggtgt attcccagta	900

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ttatattatg caatccaggc gaaggcagct cttaccagga cttcttcaat aaatcagaaa	960
atgaagccct taggtctcgg acattacaaa attcagtcct cgccaaactc acaagtcctc	1020
tctttgataa caatcaagaa gcgtctcgc aagatcaaac ttcttgtaa gtgtcatttc	1080
acaattcatc atgaatgtaa tcagggattt tcgcacagag gaaccatca cgctgaaca	1140
agcgacgaat tccatacccg tggacttgg accgaatcca ttgtacctca aactccagga	1200
cttcttcctg tccggggccag tttatcaact gaaagtccag ataagattca accacaacct	1260
ccgcaaatac ttgaatcttc acaagtgtg gatagatttg acgatcactg gatcgcacag	1320
gacattaact ggggaccgtt ttttgaaagt cttgaaaaag agactagaga tatacttgga	1380
taatttaggt ttaatttgta ttaataatgt aattagaggt ttaaatcatg tctgtatga	1440
agaatttaat tttgtatcta gtgtaattca gaaccagag gttgcaatga aattgtatta	1500
aaaaaataat atttttatta ataaaaataa catctacaat tgccaa	1546

<210> SEQ ID NO 10
 <211> LENGTH: 181
 <212> TYPE: DNA
 <213> ORGANISM: Agrobacterium
 <220> FEATURE:
 <221> NAME/KEY: promoter
 <222> LOCATION: (1)..(181)
 <223> OTHER INFORMATION: NOS promoter

<400> SEQUENCE: 10

tttctggagt ttaatgagct aagcacatac gtcagaaacc attattgcgc gttcaaaagt	60
cgccctaagg cactatcagc tagcaaatat ttcttgtaa aaatgtcca ctgacgttcc	120
ataaattccc ctccggtatcc aattagagtc tcatattcac tctcaatcca aataatctgc	180
a	181

<210> SEQ ID NO 11
 <211> LENGTH: 2259
 <212> TYPE: DNA
 <213> ORGANISM: T. ethanolicus
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(2259)
 <223> OTHER INFORMATION: dicot optimized alpha-1,6-glucosidase

<400> SEQUENCE: 11

atg tac caa aag act tct gag aag atc gtt gtt agg aac gag gga aag	48
Met Tyr Gln Lys Thr Ser Glu Lys Ile Val Val Arg Asn Glu Gly Lys	
1 5 10 15	
aag ttg gag ctt agg gtt ctc gga gat aag atc atc aac gtg ttc gtg	96
Lys Leu Glu Leu Arg Val Leu Gly Asp Lys Ile Ile Asn Val Phe Val	
20 25 30	
tcc aac aaa gaa gag aag agg aag gat aca att gct atc gag agg aaa	144
Ser Asn Lys Glu Glu Lys Arg Lys Asp Thr Ile Ala Ile Glu Arg Lys	
35 40 45	
gag tac gat act cca gag ttc tct atc tct gat gag ctt gag tct atc	192
Glu Tyr Asp Thr Pro Glu Phe Ser Ile Ser Asp Glu Leu Glu Ser Ile	
50 55 60	
ctc att gag act aac tcc ctc aag gtg aag atc aac aag aac gat ctt	240
Leu Ile Glu Thr Asn Ser Leu Lys Val Lys Ile Asn Lys Asn Asp Leu	
65 70 75 80	
tct gtg tcc ttc ttg gat aag aac gga aac atc atc aac gag gat tac	288
Ser Val Ser Phe Leu Asp Lys Asn Gly Asn Ile Ile Asn Glu Asp Tyr	
85 90 95	
aat ggt gga gct aag ttc aac gag act gat gtt agg tgc tac aag aag	336

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Asn	Gly	Gly	Ala	Lys	Phe	Asn	Glu	Thr	Asp	Val	Arg	Cys	Tyr	Lys	Lys	
			100					105					110			
ttg	aga	gag	gat	cac	ttt	tac	gga	ttt	gga	gag	aag	gct	gga	tac	ctt	384
Leu	Arg	Glu	Asp	His	Phe	Tyr	Gly	Phe	Gly	Glu	Lys	Ala	Gly	Tyr	Leu	
		115					120					125				
gat	aag	aag	ggt	gaa	agg	ctt	gag	atg	tgg	aac	act	gat	gag	ttc	atg	432
Asp	Lys	Lys	Gly	Glu	Arg	Leu	Glu	Met	Trp	Asn	Thr	Asp	Glu	Phe	Met	
		130				135					140					
act	cac	aac	cag	act	act	aag	ctc	ctt	tac	gag	tcc	tac	cca	ttc	ttc	480
Thr	His	Asn	Gln	Thr	Thr	Lys	Leu	Leu	Tyr	Glu	Ser	Tyr	Pro	Phe	Phe	
	145				150					155				160		
atc	gga	atg	aac	gat	tac	cac	act	tac	gga	atc	ttt	ctc	gat	aac	tcc	528
Ile	Gly	Met	Asn	Asp	Tyr	His	Thr	Tyr	Gly	Ile	Phe	Leu	Asp	Asn	Ser	
			165						170					175		
ttc	cgt	tcc	ttc	ttt	gat	atg	gga	caa	gag	tcc	caa	gag	tac	tac	ttt	576
Phe	Arg	Ser	Phe	Phe	Asp	Met	Gly	Gln	Glu	Ser	Gln	Glu	Tyr	Tyr	Phe	
			180					185					190			
ttc	gga	gct	tac	ggt	gga	caa	atg	aac	tac	tac	ttc	atc	tac	ggt	gaa	624
Phe	Gly	Ala	Tyr	Gly	Gly	Gln	Met	Asn	Tyr	Tyr	Phe	Ile	Tyr	Gly	Glu	
		195				200						205				
gat	atc	aaa	gaa	gtg	gtg	gag	aac	tac	act	tat	ctc	act	gga	agg	att	672
Asp	Ile	Lys	Glu	Val	Val	Glu	Asn	Tyr	Thr	Tyr	Leu	Thr	Gly	Arg	Ile	
	210					215					220					
tct	ctt	cca	cca	ctt	tgg	gtt	ttg	gga	aat	cag	cag	tct	agg	tac	tct	720
Ser	Leu	Pro	Pro	Leu	Trp	Val	Leu	Gly	Asn	Gln	Gln	Ser	Arg	Tyr	Ser	
	225				230					235				240		
tat	act	cca	caa	gag	agg	gtt	ttg	gag	gtt	gca	aag	act	ttc	aga	gag	768
Tyr	Thr	Pro	Gln	Arg	Val	Leu	Glu	Val	Ala	Lys	Thr	Phe	Arg	Glu		
			245					250					255			
aag	gat	atc	cct	tgc	gat	gtg	atc	tac	ctc	gat	atc	gat	tac	atg	gaa	816
Lys	Asp	Ile	Pro	Cys	Asp	Val	Ile	Tyr	Leu	Asp	Ile	Asp	Tyr	Met	Glu	
		260					265					270				
gga	tac	cgt	gtt	ttc	act	tgg	aac	aaa	gag	act	ttc	aag	aac	cac	aaa	864
Gly	Tyr	Arg	Val	Phe	Thr	Trp	Asn	Lys	Glu	Thr	Phe	Lys	Asn	His	Lys	
		275				280					285					
gag	atg	ctt	aag	cag	ctc	aaa	gag	atg	ggt	ttc	aag	gtt	gtg	act	atc	912
Glu	Met	Leu	Lys	Gln	Leu	Lys	Glu	Met	Gly	Phe	Lys	Val	Val	Thr	Ile	
	290				295					300						
gtt	gat	cca	ggt	gtt	aag	agg	gat	tac	gat	tac	cat	gtg	tac	cgt	gaa	960
Val	Asp	Pro	Gly	Val	Lys	Arg	Asp	Tyr	Asp	Tyr	His	Val	Tyr	Arg	Glu	
	305				310				315					320		
ggt	att	gag	aag	gga	tac	ttc	gtg	aag	gat	aag	tac	gga	atc	act	tat	1008
Gly	Ile	Glu	Lys	Gly	Tyr	Phe	Val	Lys	Asp	Lys	Tyr	Gly	Ile	Thr	Tyr	
			325					330					335			
gtg	gga	aaa	gtt	tgg	cct	ggc	gag	gct	tgt	ttt	cca	gat	ttc	ctc	caa	1056
Val	Gly	Lys	Val	Trp	Pro	Gly	Glu	Ala	Cys	Phe	Pro	Asp	Phe	Leu	Gln	
		340				345							350			
gag	gaa	gtt	aga	tat	tgg	tgg	gga	gaa	aag	cac	aga	gag	ttc	atc	aac	1104
Glu	Glu	Val	Arg	Tyr	Trp	Trp	Gly	Glu	Lys	His	Arg	Glu	Phe	Ile	Asn	
		355				360						365				
gac	gga	atc	gat	ggt	atc	tgg	aac	gat	atg	aac	gag	cca	gct	gtt	ttt	1152
Asp	Gly	Ile	Asp	Gly	Ile	Trp	Asn	Asp	Met	Asn	Glu	Pro	Ala	Val	Phe	
	370				375						380					
gaa	act	cca	act	aag	act	atg	cca	gag	gat	aac	atc	cac	att	ctc	gat	1200
Glu	Thr	Pro	Thr	Lys	Thr	Met	Pro	Glu	Asp	Asn	Ile	His	Ile	Leu	Asp	
	385				390					395				400		
ggt	gaa	aag	gtt	ctc	cac	aaa	gag	gct	cat	aac	gtt	tac	gct	aac	tac	1248
Gly	Glu	Lys	Val	Leu	His	Lys	Glu	Ala	His	Asn	Val	Tyr	Ala	Asn	Tyr	
			405					410					415			

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atg gct atg gct act agg gat gga ttt ctc agg att agg cca aat gag	1296
Met Ala Met Ala Thr Arg Asp Gly Phe Leu Arg Ile Arg Pro Asn Glu	
420 425 430	
agg cca ttt gtg ctt act agg gct gct ttc tct gga att cag cgt tat	1344
Arg Pro Phe Val Leu Thr Arg Ala Ala Phe Ser Gly Ile Gln Arg Tyr	
435 440 445	
gct gct atg tgg act ggt gat aac aga tct ctt tac gag cac ctc ctt	1392
Ala Ala Met Trp Thr Gly Asp Asn Arg Ser Leu Tyr Glu His Leu Leu	
450 455 460	
atg atg atg cct atg ctc atg aac atc gga ctt tct gga caa cca ttc	1440
Met Met Met Pro Met Leu Met Asn Ile Gly Leu Ser Gly Gln Pro Phe	
465 470 475 480	
gtt ggt gct gat gtt gga gga ttt gag ggc gat tgc cac gag gaa ctt	1488
Val Gly Ala Asp Val Gly Gly Phe Glu Gly Asp Cys His Glu Glu Leu	
485 490 495	
ttc att aga tgg atc gag gct gct gtt ttt act cca ttc ctt agg gtg	1536
Phe Ile Arg Trp Ile Glu Ala Ala Val Phe Thr Pro Phe Leu Arg Val	
500 505 510	
cac tct gct att gga act aag gat caa gag cct tgg tct ttt gga aag	1584
His Ser Ala Ile Gly Thr Lys Asp Gln Glu Pro Trp Ser Phe Gly Lys	
515 520 525	
agg gct gag gat att tcc cgt aag tac atc aag atg cgt tac gag ctt	1632
Arg Ala Glu Asp Ile Ser Arg Lys Tyr Ile Lys Met Arg Tyr Glu Leu	
530 535 540	
ctt cca tac ctt tac gat ctc ttc tac att gct tcc caa aag gga tac	1680
Leu Pro Tyr Leu Tyr Asp Leu Phe Tyr Ile Ala Ser Gln Lys Gly Tyr	
545 550 555 560	
cca att atg agg cca ctt gtg ttt gag tac cag aag gat gag aac act	1728
Pro Ile Met Arg Pro Leu Val Phe Glu Tyr Gln Lys Asp Glu Asn Thr	
565 570 575	
cac aag atc tac gat gag ttt atg ttc gga gag gga ctt ctt gtt gct	1776
His Lys Ile Tyr Asp Glu Phe Met Phe Gly Glu Gly Leu Leu Val Ala	
580 585 590	
cca gtg tac ctt cca tct aaa gag cgt aga gag gtt tac ctt cca gag	1824
Pro Val Tyr Leu Pro Ser Lys Glu Arg Arg Glu Val Tyr Leu Pro Glu	
595 600 605	
gga atc tgg tat gat tac tgg act gga aag gga ttc aag gga aag aac	1872
Gly Ile Trp Tyr Asp Tyr Trp Thr Gly Lys Gly Phe Lys Gly Lys Asn	
610 615 620	
tac tac ctt gtg gat gct cca att gag gtt atc cca ctc ttt gtg aaa	1920
Tyr Tyr Leu Val Asp Ala Pro Ile Glu Val Ile Pro Leu Phe Val Lys	
625 630 635 640	
gag ggt gga att ctt ctt aag cag cag cca cag tct ttt att gga gag	1968
Glu Gly Gly Ile Leu Leu Lys Gln Gln Pro Gln Ser Phe Ile Gly Glu	
645 650 655	
aag aag ctc gag gaa ctt act gtt gag atc tac aag gga aaa gag gga	2016
Lys Lys Leu Glu Glu Leu Thr Val Glu Ile Tyr Lys Gly Lys Glu Gly	
660 665 670	
cat tac ctc cat tat gag gat gat gga aag tcc ttc gat tac act aag	2064
His Tyr Leu His Tyr Glu Asp Asp Gly Lys Ser Phe Asp Tyr Thr Lys	
675 680 685	
ggc gtg tac aac ctc ttc gat atc tca ttc tgc tac aaa gag gga agg	2112
Gly Val Tyr Asn Leu Phe Asp Ile Ser Phe Cys Tyr Lys Glu Gly Arg	
690 695 700	
atg gat atc aag ttc gat aag atc cac ttc gga tac gat aag ggt gtt	2160
Met Asp Ile Lys Phe Asp Lys Ile His Phe Gly Tyr Asp Lys Gly Val	
705 710 715 720	
aag aag tac aag ttc atc ttc aag aac ttc gat gat atc aaa gag atc	2208
Lys Lys Tyr Lys Phe Ile Phe Lys Asn Phe Asp Asp Ile Lys Glu Ile	
725 730 735	

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aag atc aac ggc gag aag gtt gag aaa gag tct tgc gag att gag ctt      2256
Lys Ile Asn Gly Glu Lys Val Glu Lys Glu Ser Cys Glu Ile Glu Leu
              740              745              750

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taa                                                                    2259

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<210> SEQ ID NO 12
<211> LENGTH: 752
<212> TYPE: PRT
<213> ORGANISM: T. ethanolicus

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<400> SEQUENCE: 12

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Met Tyr Gln Lys Thr Ser Glu Lys Ile Val Val Arg Asn Glu Gly Lys
1              5              10              15

Lys Leu Glu Leu Arg Val Leu Gly Asp Lys Ile Ile Asn Val Phe Val
20              25              30

Ser Asn Lys Glu Glu Lys Arg Lys Asp Thr Ile Ala Ile Glu Arg Lys
35              40              45

Glu Tyr Asp Thr Pro Glu Phe Ser Ile Ser Asp Glu Leu Glu Ser Ile
50              55              60

Leu Ile Glu Thr Asn Ser Leu Lys Val Lys Ile Asn Lys Asn Asp Leu
65              70              75              80

Ser Val Ser Phe Leu Asp Lys Asn Gly Asn Ile Ile Asn Glu Asp Tyr
85              90              95

Asn Gly Gly Ala Lys Phe Asn Glu Thr Asp Val Arg Cys Tyr Lys Lys
100              105              110

Leu Arg Glu Asp His Phe Tyr Gly Phe Gly Glu Lys Ala Gly Tyr Leu
115              120              125

Asp Lys Lys Gly Glu Arg Leu Glu Met Trp Asn Thr Asp Glu Phe Met
130              135              140

Thr His Asn Gln Thr Thr Lys Leu Leu Tyr Glu Ser Tyr Pro Phe Phe
145              150              155              160

Ile Gly Met Asn Asp Tyr His Thr Tyr Gly Ile Phe Leu Asp Asn Ser
165              170              175

Phe Arg Ser Phe Phe Asp Met Gly Gln Glu Ser Gln Glu Tyr Tyr Phe
180              185              190

Phe Gly Ala Tyr Gly Gly Gln Met Asn Tyr Tyr Phe Ile Tyr Gly Glu
195              200              205

Asp Ile Lys Glu Val Val Glu Asn Tyr Thr Tyr Leu Thr Gly Arg Ile
210              215              220

Ser Leu Pro Pro Leu Trp Val Leu Gly Asn Gln Gln Ser Arg Tyr Ser
225              230              235              240

Tyr Thr Pro Gln Glu Arg Val Leu Glu Val Ala Lys Thr Phe Arg Glu
245              250              255

Lys Asp Ile Pro Cys Asp Val Ile Tyr Leu Asp Ile Asp Tyr Met Glu
260              265              270

Gly Tyr Arg Val Phe Thr Trp Asn Lys Glu Thr Phe Lys Asn His Lys
275              280              285

Glu Met Leu Lys Gln Leu Lys Glu Met Gly Phe Lys Val Val Thr Ile
290              295              300

Val Asp Pro Gly Val Lys Arg Asp Tyr Asp Tyr His Val Tyr Arg Glu
305              310              315              320

Gly Ile Glu Lys Gly Tyr Phe Val Lys Asp Lys Tyr Gly Ile Thr Tyr
325              330              335

Val Gly Lys Val Trp Pro Gly Glu Ala Cys Phe Pro Asp Phe Leu Gln

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340							345					350				
Glu	Glu	Val	Arg	Tyr	Trp	Trp	Gly	Glu	Lys	His	Arg	Glu	Phe	Ile	Asn	
		355					360					365				
Asp	Gly	Ile	Asp	Gly	Ile	Trp	Asn	Asp	Met	Asn	Glu	Pro	Ala	Val	Phe	
	370					375					380					
Glu	Thr	Pro	Thr	Lys	Thr	Met	Pro	Glu	Asp	Asn	Ile	His	Ile	Leu	Asp	
	385				390					395					400	
Gly	Glu	Lys	Val	Leu	His	Lys	Glu	Ala	His	Asn	Val	Tyr	Ala	Asn	Tyr	
			405						410					415		
Met	Ala	Met	Ala	Thr	Arg	Asp	Gly	Phe	Leu	Arg	Ile	Arg	Pro	Asn	Glu	
			420					425					430			
Arg	Pro	Phe	Val	Leu	Thr	Arg	Ala	Ala	Phe	Ser	Gly	Ile	Gln	Arg	Tyr	
		435					440					445				
Ala	Ala	Met	Trp	Thr	Gly	Asp	Asn	Arg	Ser	Leu	Tyr	Glu	His	Leu	Leu	
	450					455						460				
Met	Met	Met	Pro	Met	Leu	Met	Asn	Ile	Gly	Leu	Ser	Gly	Gln	Pro	Phe	
	465				470					475					480	
Val	Gly	Ala	Asp	Val	Gly	Gly	Phe	Glu	Gly	Asp	Cys	His	Glu	Glu	Leu	
			485						490					495		
Phe	Ile	Arg	Trp	Ile	Glu	Ala	Ala	Val	Phe	Thr	Pro	Phe	Leu	Arg	Val	
			500					505					510			
His	Ser	Ala	Ile	Gly	Thr	Lys	Asp	Gln	Glu	Pro	Trp	Ser	Phe	Gly	Lys	
		515					520					525				
Arg	Ala	Glu	Asp	Ile	Ser	Arg	Lys	Tyr	Ile	Lys	Met	Arg	Tyr	Glu	Leu	
	530					535					540					
Leu	Pro	Tyr	Leu	Tyr	Asp	Leu	Phe	Tyr	Ile	Ala	Ser	Gln	Lys	Gly	Tyr	
	545				550					555					560	
Pro	Ile	Met	Arg	Pro	Leu	Val	Phe	Glu	Tyr	Gln	Lys	Asp	Glu	Asn	Thr	
			565						570					575		
His	Lys	Ile	Tyr	Asp	Glu	Phe	Met	Phe	Gly	Glu	Gly	Leu	Leu	Val	Ala	
		580						585					590			
Pro	Val	Tyr	Leu	Pro	Ser	Lys	Glu	Arg	Arg	Glu	Val	Tyr	Leu	Pro	Glu	
		595					600					605				
Gly	Ile	Trp	Tyr	Asp	Tyr	Trp	Thr	Gly	Lys	Gly	Phe	Lys	Gly	Lys	Asn	
	610					615					620					
Tyr	Tyr	Leu	Val	Asp	Ala	Pro	Ile	Glu	Val	Ile	Pro	Leu	Phe	Val	Lys	
	625				630					635					640	
Glu	Gly	Gly	Ile	Leu	Leu	Lys	Gln	Gln	Pro	Gln	Ser	Phe	Ile	Gly	Glu	
			645						650					655		
Lys	Lys	Leu	Glu	Glu	Leu	Thr	Val	Glu	Ile	Tyr	Lys	Gly	Lys	Glu	Gly	
		660						665					670			
His	Tyr	Leu	His	Tyr	Glu	Asp	Asp	Gly	Lys	Ser	Phe	Asp	Tyr	Thr	Lys	
		675					680					685				
Gly	Val	Tyr	Asn	Leu	Phe	Asp	Ile	Ser	Phe	Cys	Tyr	Lys	Glu	Gly	Arg	
	690					695					700					
Met	Asp	Ile	Lys	Phe	Asp	Lys	Ile	His	Phe	Gly	Tyr	Asp	Lys	Gly	Val	
	705				710					715					720	
Lys	Lys	Tyr	Lys	Phe	Ile	Phe	Lys	Asn	Phe	Asp	Asp	Ile	Lys	Glu	Ile	
			725						730					735		
Lys	Ile	Asn	Gly	Glu	Lys	Val	Glu	Lys	Glu	Ser	Cys	Glu	Ile	Glu	Leu	
		740						745					750			

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<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: soybean
<220> FEATURE:
<221> NAME/KEY: targeting
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION: ER targeting sequence

<400> SEQUENCE: 13

Met Ala Lys Leu Val Phe Ser Leu Cys Phe Leu Leu Phe Ser Gly Cys
1             5             10             15

Cys Phe Ala

<210> SEQ ID NO 14
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: Erwinia carotovora
<220> FEATURE:
<221> NAME/KEY: enzyme
<222> LOCATION: (1)..(566)
<223> OTHER INFORMATION: sucrose isomerase YP 049947

<400> SEQUENCE: 14

Met Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys
1             5             10             15

Glu Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp
20             25             30

Gly Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr
35             40             45

Leu Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp
50             55             60

Ser Pro Asn Thr Asp Asn Gly Tyr Asp Ile Arg Asp Tyr Arg Lys Ile
65             70             75             80

Met Lys Glu Tyr Gly Thr Met Asp Asp Phe Asp Arg Leu Ile Ala Glu
85             90             95

Met Lys Lys Arg Asp Met Arg Leu Met Ile Asp Val Val Val Asn His
100            105            110

Thr Ser Asp Glu His Glu Trp Phe Val Glu Ser Lys Lys Ser Lys Asp
115            120            125

Asn Pro Tyr Arg Asp Tyr Tyr Ile Trp Arg Asp Gly Lys Asp Gly Thr
130            135            140

Gln Pro Asn Asn Tyr Pro Ser Phe Phe Gly Gly Ser Ala Trp Gln Lys
145            150            155            160

Asp Asn Ala Thr Gln Gln Tyr Tyr Leu His Tyr Phe Gly Val Gln Gln
165            170            175

Pro Asp Leu Asn Trp Asp Asn Pro Lys Val Arg Glu Glu Val Tyr Asp
180            185            190

Met Leu Arg Phe Trp Ile Asp Lys Gly Val Ser Gly Leu Arg Met Asp
195            200            205

Thr Val Ala Thr Phe Ser Lys Asn Pro Ala Phe Pro Asp Leu Thr Pro
210            215            220

Lys Gln Leu Gln Asn Phe Ala Tyr Thr Tyr Thr Gln Gly Pro Asn Leu
225            230            235            240

His Arg Tyr Ile Gln Glu Met His Gln Lys Val Leu Ala Lys Tyr Asp
245            250            255

Val Val Ser Ala Gly Glu Ile Phe Gly Val Pro Leu Glu Glu Ala Ala
260            265            270

Pro Phe Ile Asp Gln Arg Arg Lys Glu Leu Asp Met Ala Phe Ser Phe

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275				280				285							
Asp	Leu	Ile	Arg	Leu	Asp	Arg	Ala	Val	Glu	Glu	Arg	Trp	Arg	Arg	Asn
290				295				300							
Asp	Trp	Thr	Leu	Ser	Gln	Phe	Arg	Gln	Ile	Asn	Asn	Arg	Leu	Val	Asp
305				310				315				320			
Met	Ala	Gly	Gln	His	Gly	Trp	Asn	Thr	Phe	Phe	Leu	Ser	Asn	His	Asp
				325				330				335			
Asn	Pro	Arg	Ala	Val	Ser	His	Phe	Gly	Asp	Asp	Arg	Pro	Glu	Trp	Arg
				340				345				350			
Thr	Arg	Ser	Ala	Lys	Ala	Leu	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Arg	Ala
				355				360				365			
Thr	Pro	Phe	Ile	Tyr	Gln	Gly	Asp	Glu	Leu	Gly	Met	Thr	Asn	Tyr	Pro
				370				375				380			
Phe	Thr	Ser	Leu	Ser	Glu	Phe	Asp	Asp	Ile	Glu	Val	Lys	Gly	Phe	Trp
385				390				395				400			
Gln	Asp	Phe	Val	Glu	Thr	Gly	Lys	Val	Lys	Pro	Asp	Val	Phe	Leu	Glu
				405				410				415			
Asn	Val	Lys	Gln	Thr	Ser	Arg	Asp	Asn	Ser	Arg	Thr	Pro	Phe	Gln	Trp
				420				425				430			
Ser	Asn	Thr	Ala	Gln	Ala	Gly	Phe	Thr	Thr	Gly	Thr	Pro	Trp	Phe	Arg
				435				440				445			
Ile	Asn	Pro	Asn	Tyr	Lys	Asn	Ile	Asn	Ala	Glu	Glu	Gln	Thr	Gln	Asn
450				455				460							
Pro	Asp	Ser	Ile	Phe	His	Phe	Tyr	Arg	Gln	Leu	Ile	Glu	Leu	Arg	His
465				470				475				480			
Ala	Thr	Pro	Ala	Phe	Thr	Tyr	Gly	Thr	Tyr	Gln	Asp	Leu	Asp	Pro	Asn
				485				490				495			
Asn	Asn	Glu	Val	Leu	Ala	Tyr	Thr	Arg	Glu	Leu	Asn	Gln	Gln	Arg	Tyr
500				505				510							
Leu	Val	Val	Val	Asn	Phe	Lys	Glu	Lys	Pro	Val	His	Tyr	Val	Leu	Pro
515				520				525							
Lys	Thr	Leu	Ser	Ile	Lys	Gln	Ser	Leu	Leu	Glu	Ser	Gly	Gln	Lys	Asp
530				535				540							
Lys	Val	Glu	Pro	Asn	Ala	Thr	Thr	Leu	Glu	Leu	Gln	Pro	Trp	Gln	Ser
545				550				555				560			
Gly	Ile	Tyr	Gln	Leu	Asn										
565															

<400> SEQUENCE: 15

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<210> SEQ ID NO 16
<211> LENGTH: 1701
<212> TYPE: DNA
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: artificial sequence
<220> FEATURE:
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<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1701)
<223> OTHER INFORMATION: sucrose isomerase dicot optimized

<400> SEQUENCE: 16

atg gtt gct gtt aac gat ggt gtt tct gct cat cca gtt tgg tgg aaa      48
Met Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys
1          5          10          15

gag gct gtt ttc tac caa gtt tac cca cgt tct ttc aag gat tcc gat      96
Glu Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp
          20          25          30

ggt gat gga att gga gat ctc aag gga ctt act gag aag ctc gat tac     144
Gly Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr
          35          40          45

ctt aag gct ctc ggt att aac gct atc tgg atc aac cca cac tac gat     192
Leu Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp
          50          55          60

tct cca aac act gat aac gga tac gat atc agg gat tac cgt aaa atc     240
Ser Pro Asn Thr Asp Asn Gly Tyr Asp Ile Arg Asp Tyr Arg Lys Ile
65          70          75          80

atg aag gaa tac gga act atg gat gat ttc gat agg ctt atc gct gaa     288
Met Lys Glu Tyr Thr Met Asp Asp Phe Asp Arg Leu Ile Ala Glu
          85          90          95

atg aag aaa agg gat atg agg ctc atg att gat gtt gtg gtg aac cac     336
Met Lys Lys Arg Asp Met Arg Leu Met Ile Asp Val Val Val Asn His
          100          105          110

act tct gat gag cat gag tgg ttc gtt gag tct aag aag tcc aag gat     384
Thr Ser Asp Glu His Glu Trp Phe Val Glu Ser Lys Lys Ser Lys Asp
          115          120          125

aac cca tac cgt gat tac tac atc tgg cgt gat gga aag gat gga act     432
Asn Pro Tyr Arg Asp Tyr Tyr Ile Trp Arg Asp Gly Lys Asp Gly Thr
          130          135          140

cag cca aat aac tac cca tct ttc ttc ggt gga tct gct tgg caa aag     480
Gln Pro Asn Asn Tyr Pro Ser Phe Phe Gly Gly Ser Ala Trp Gln Lys
          145          150          155          160

gat aat gct act cag cag tac tac ctt cac tac ttc gga gtt caa cag     528
Asp Asn Ala Thr Gln Gln Tyr Tyr Leu His Tyr Phe Gly Val Gln Gln
          165          170          175

cca gat ctc aat tgg gat aac cca aaa gtt agg gaa gag gtg tac gat     576
Pro Asp Leu Asn Trp Asp Asn Pro Lys Val Arg Glu Glu Val Tyr Asp
          180          185          190

atg ctt agg ttc tgg atc gat aag ggt gtt agt gga ctc aga atg gat     624
Met Leu Arg Phe Trp Ile Asp Lys Gly Val Ser Gly Leu Arg Met Asp
          195          200          205

act gtg gct act ttc tct aag aat cca gct ttc cca gat ctt act cca     672
Thr Val Ala Thr Phe Ser Lys Asn Pro Ala Phe Pro Asp Leu Thr Pro
          210          215          220

aag cag ctt cag aac ttc gct tac act tac act cag gga cca aat ctt     720
Lys Gln Leu Gln Asn Phe Ala Tyr Thr Tyr Thr Gln Gly Pro Asn Leu
          225          230          235          240

cac cgt tac atc caa gag atg cac caa aag gtt ctc gct aag tac gat     768
His Arg Tyr Ile Gln Glu Met His Gln Lys Val Leu Ala Lys Tyr Asp
          245          250          255

gtt gtt tcc gct ggt gaa att ttc gga gtg cca ctt gaa gaa gct gct     816
Val Val Ser Ala Gly Glu Ile Phe Gly Val Pro Leu Glu Glu Ala Ala
          260          265          270

cca ttc att gat cag agg cgt aaa gaa ctc gat atg gct ttc tcc ttc     864
Pro Phe Ile Asp Gln Arg Arg Lys Glu Leu Asp Met Ala Phe Ser Phe
          275          280          285

gat ctt atc cgt ctt gat agg gct gtt gaa gaa agg tgg agg cgt aat     912

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Asp 290	Leu	Ile	Arg	Leu	Asp 295	Arg	Ala	Val	Glu	Glu	Arg 300	Trp	Arg	Arg	Asn	
gat	tg	act	ttg	tcc	cag	ttc	agg	cag	att	aac	aac	agg	ctt	gtg	gat	960
Asp	Trp	Thr	Leu	Ser	Gln	Phe	Arg	Gln	Ile	Asn	Asn	Arg	Leu	Val	Asp	
305					310					315					320	
atg	gct	gga	caa	cat	gga	tg	aat	act	ttc	ttc	ctc	tcc	aac	cat	gat	1008
Met	Ala	Gly	Gln	His	Gly	Trp	Asn	Thr	Phe	Phe	Leu	Ser	Asn	His	Asp	
				325					330					335		
aat	cca	agg	gct	gtt	tct	cat	ttc	gga	gat	gat	aga	cca	gag	tg	aga	1056
Asn	Pro	Arg	Ala	Val	Ser	His	Phe	Gly	Asp	Asp	Arg	Pro	Glu	Trp	Arg	
			340					345					350			
act	aga	tct	gct	aag	gct	ctt	gct	act	ctt	gct	ctt	act	caa	agg	gct	1104
Thr	Arg	Ser	Ala	Lys	Ala	Leu	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Arg	Ala	
			355				360					365				
act	cca	ttc	atc	tat	cag	gg	gat	gag	ctt	gga	atg	act	aac	tac	cca	1152
Thr	Pro	Phe	Ile	Tyr	Gln	Gly	Asp	Glu	Leu	Gly	Met	Thr	Asn	Tyr	Pro	
	370					375					380					
ttc	act	tca	ctt	tcc	gag	ttc	gat	gat	att	gag	gtg	aag	gga	ttc	tg	1200
Phe	Thr	Ser	Leu	Ser	Glu	Phe	Asp	Asp	Ile	Glu	Val	Lys	Gly	Phe	Trp	
385					390					395				400		
caa	gat	ttt	gtg	gag	act	gga	aag	gtt	aag	cca	gat	gtg	ttc	ctt	gag	1248
Gln	Asp	Phe	Val	Glu	Thr	Gly	Lys	Val	Lys	Pro	Asp	Val	Phe	Leu	Glu	
				405					410					415		
aac	gtg	aag	caa	act	tct	agg	gat	aac	tcc	agg	act	cca	ttc	caa	tg	1296
Asn	Val	Lys	Gln	Thr	Ser	Arg	Asp	Asn	Ser	Arg	Thr	Pro	Phe	Gln	Trp	
			420					425					430			
tct	aat	act	gct	cag	gct	gga	ttc	act	act	gga	aca	cct	tg	ttt	agg	1344
Ser	Asn	Thr	Ala	Gln	Ala	Gly	Phe	Thr	Thr	Gly	Thr	Pro	Trp	Phe	Arg	
			435				440					445				
atc	aac	cct	aac	tac	aag	aac	atc	aac	gct	gag	gaa	caa	act	cag	aac	1392
Ile	Asn	Pro	Asn	Tyr	Lys	Asn	Ile	Asn	Ala	Glu	Glu	Gln	Thr	Gln	Asn	
			450				455					460				
cca	gat	tcc	atc	ttc	cat	ttc	tac	cgt	cag	ttg	att	gaa	ctt	agg	cat	1440
Pro	Asp	Ser	Ile	Phe	His	Phe	Tyr	Arg	Gln	Leu	Ile	Glu	Leu	Arg	His	
					470					475					480	
gct	act	cca	gct	ttt	act	tac	gga	act	tac	cag	gat	ctt	gat	cca	aac	1488
Ala	Thr	Pro	Ala	Phe	Thr	Tyr	Gly	Thr	Tyr	Gln	Asp	Leu	Asp	Pro	Asn	
				485				490						495		
aac	aac	gag	gtt	ctc	gct	tac	act	aga	gag	ctt	aac	cag	cag	aga	tac	1536
Asn	Asn	Glu	Val	Leu	Ala	Tyr	Thr	Arg	Glu	Leu	Asn	Gln	Gln	Arg	Tyr	
			500					505					510			
ctt	gtt	gtt	gtg	aac	ttc	aaa	gag	aag	cca	gtt	cac	tac	gtt	ctt	cca	1584
Leu	Val	Val	Val	Asn	Phe	Lys	Glu	Lys	Pro	Val	His	Tyr	Val	Leu	Pro	
			515				520					525				
aag	act	ctt	tcc	att	aag	cag	tct	ttg	ctt	gag	tct	gga	cag	aag	gat	1632
Lys	Thr	Leu	Ser	Ile	Lys	Gln	Ser	Leu	Leu	Glu	Ser	Gly	Gln	Lys	Asp	
			530				535				540					
aag	gtt	gag	cca	aac	gct	act	act	ctt	gag	ctt	caa	cct	tg	caa	tct	1680
Lys	Val	Glu	Pro	Asn	Ala	Thr	Thr	Leu	Glu	Leu	Gln	Pro	Trp	Gln	Ser	
				545			550			555				560		
gga	atc	tac	cag	ctc	aac	tga										1701
Gly	Ile	Tyr	Gln	Leu	Asn											
				565												

<210> SEQ ID NO 17

<211> LENGTH: 566

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

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<400> SEQUENCE: 17

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Met Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys
1      5      10      15
Glu Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp
20      25      30
Gly Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr
35      40      45
Leu Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp
50      55      60
Ser Pro Asn Thr Asp Asn Gly Tyr Asp Ile Arg Asp Tyr Arg Lys Ile
65      70      75      80
Met Lys Glu Tyr Gly Thr Met Asp Asp Phe Asp Arg Leu Ile Ala Glu
85      90      95
Met Lys Lys Arg Asp Met Arg Leu Met Ile Asp Val Val Val Asn His
100     105     110
Thr Ser Asp Glu His Glu Trp Phe Val Glu Ser Lys Lys Ser Lys Asp
115     120     125
Asn Pro Tyr Arg Asp Tyr Tyr Ile Trp Arg Asp Gly Lys Asp Gly Thr
130     135     140
Gln Pro Asn Asn Tyr Pro Ser Phe Phe Gly Gly Ser Ala Trp Gln Lys
145     150     155     160
Asp Asn Ala Thr Gln Gln Tyr Tyr Leu His Tyr Phe Gly Val Gln Gln
165     170     175
Pro Asp Leu Asn Trp Asp Asn Pro Lys Val Arg Glu Glu Val Tyr Asp
180     185     190
Met Leu Arg Phe Trp Ile Asp Lys Gly Val Ser Gly Leu Arg Met Asp
195     200     205
Thr Val Ala Thr Phe Ser Lys Asn Pro Ala Phe Pro Asp Leu Thr Pro
210     215     220
Lys Gln Leu Gln Asn Phe Ala Tyr Thr Tyr Thr Gln Gly Pro Asn Leu
225     230     235     240
His Arg Tyr Ile Gln Glu Met His Gln Lys Val Leu Ala Lys Tyr Asp
245     250     255
Val Val Ser Ala Gly Glu Ile Phe Gly Val Pro Leu Glu Glu Ala Ala
260     265     270
Pro Phe Ile Asp Gln Arg Arg Lys Glu Leu Asp Met Ala Phe Ser Phe
275     280     285
Asp Leu Ile Arg Leu Asp Arg Ala Val Glu Glu Arg Trp Arg Arg Asn
290     295     300
Asp Trp Thr Leu Ser Gln Phe Arg Gln Ile Asn Asn Arg Leu Val Asp
305     310     315     320
Met Ala Gly Gln His Gly Trp Asn Thr Phe Phe Leu Ser Asn His Asp
325     330     335
Asn Pro Arg Ala Val Ser His Phe Gly Asp Asp Arg Pro Glu Trp Arg
340     345     350
Thr Arg Ser Ala Lys Ala Leu Ala Thr Leu Ala Leu Thr Gln Arg Ala
355     360     365
Thr Pro Phe Ile Tyr Gln Gly Asp Glu Leu Gly Met Thr Asn Tyr Pro
370     375     380
Phe Thr Ser Leu Ser Glu Phe Asp Asp Ile Glu Val Lys Gly Phe Trp
385     390     395     400
Gln Asp Phe Val Glu Thr Gly Lys Val Lys Pro Asp Val Phe Leu Glu
405     410     415

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Asn Val Lys Gln Thr Ser Arg Asp Asn Ser Arg Thr Pro Phe Gln Trp
 420 425 430
 Ser Asn Thr Ala Gln Ala Gly Phe Thr Thr Gly Thr Pro Trp Phe Arg
 435 440 445
 Ile Asn Pro Asn Tyr Lys Asn Ile Asn Ala Glu Glu Gln Thr Gln Asn
 450 455 460
 Pro Asp Ser Ile Phe His Phe Tyr Arg Gln Leu Ile Glu Leu Arg His
 465 470 475 480
 Ala Thr Pro Ala Phe Thr Tyr Gly Thr Tyr Gln Asp Leu Asp Pro Asn
 485 490 495
 Asn Asn Glu Val Leu Ala Tyr Thr Arg Glu Leu Asn Gln Gln Arg Tyr
 500 505 510
 Leu Val Val Val Asn Phe Lys Glu Lys Pro Val His Tyr Val Leu Pro
 515 520 525
 Lys Thr Leu Ser Ile Lys Gln Ser Leu Leu Glu Ser Gly Gln Lys Asp
 530 535 540
 Lys Val Glu Pro Asn Ala Thr Thr Leu Glu Leu Gln Pro Trp Gln Ser
 545 550 555 560
 Gly Ile Tyr Gln Leu Asn
 565

<210> SEQ ID NO 18
 <211> LENGTH: 1993
 <212> TYPE: DNA
 <213> ORGANISM: Zea mays
 <220> FEATURE:
 <221> NAME/KEY: promoter
 <222> LOCATION: (1)..(1993)
 <223> OTHER INFORMATION: maize UBQ promoter
 <400> SEQUENCE: 18

ctgcagtgcac ggcgtgacccg gtcgtgcccc tctctagaga taatgagcat tgcattgtcta	60
agttataaaa aattaccaca tatttttttt gtcacacttg tttgaagtgc agtttatcta	120
tctttataca tatatttaaa ctttactcta cgaataatat aatctatagt actacaataa	180
tatcagtgtt tttagaatac atataaatga acagtttagac atgggtctaaa ggacaattga	240
gtattttgac aacaggactc tacagtttta tcttttttagt gtgcattgtgt tctccttttt	300
ttttgcaaat agcttcacct atataatact tcatccattt tattagtaca tccatttagg	360
gtttagggtt aatgggtttt atagactaat ttttttagta catctatttt attctatttt	420
agcctctaaa ttaagaaaac taaaactcta ttttagtttt tttatttaaat aatttagata	480
taaaatagaa taaaataaag tgactaaaaa ttaaacaat accctttaag aaattaaaaa	540
aactaaggaa acattttttc tgtttcgagt agataatgcc agcctgttaa acgcgcgcga	600
cgagtctaac ggacaccaac cagcgaacca gcagcgtcgc gtcggggccaa gcgaagcaga	660
cggcacggca tctctgtcgc tgccctctgga cccctctcga gagttccgct ccaccgttgg	720
acttgctccg ctgtcggcat ccagaaattg cgtggcggag cggcagacgt gagccggcac	780
ggcaggcggc ctctctctcc tctcacggca ccggcagcta cgggggatcc ctttccacc	840
gctctctcgc tttcccttcc tcgcccgcgc taataaatag acacccctc caccacctct	900
ttccccaacc tcgtgttgtt cggagcgcac acacacacaa ccagatctcc cccaaatcca	960
cccgctggca cctccgcttc aaggtaacgc gctcgtctcc ccccccccc cctctctacc	1020
ttctctagat cggcggtccg gtccatagtt agggcccggt agttctactt ctgttcattg	1080

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ttgtgttaga tccgtgtttg tgtagatcc gtgctgtag cggtcgtaca cggatgcgac 1140
ctgtacgtca gacacgttct gattgctaac ttgccagtgt ttctctttgg ggaatcctgg 1200
gatggctcta gccgttcgcg agacgggagc gatttcatga ttttttttgg ttcgttgcat 1260
agggtttggg ttgccctttt cctttatttc aatatatgcc gtgcacttgt ttgtcgggtc 1320
atcttttcat gctttttttt gtcttggttg tgatgatgtg gtctggttgg gcggtcgttc 1380
tagatcggag tagaattctg tttcaacta cctggtggat ttattaattt tggatctgta 1440
tgtgtgtgcc atacatattc atagttacga attgaagatg atggatggaa atactgatct 1500
aggataggta tacatgttga tgcgggtttt actgatgcat atacagagat gctttttgtt 1560
cgcttggttg tgatgatgtg gtgtggttgg gcggtcgttc attcgttcta gatcggagta 1620
gaatactgtt tcaaaactacc tgggtgtattt attaatTTTg gaactgtatg tgtgtgtcat 1680
acatcttcat agttacgagt ttaagatgga tggaaatacc gatctaggat aggtatacat 1740
gttgatgtgg gttttactga tgcataata tgatggcata tgcagcatct attcatatgc 1800
tctaaccttg agtacctatc tattataata aacaagtatg tttataatt attttgatct 1860
tgatatactt ggatgatggc atatgcagca gctatatgtg gattttttta gccctgcctt 1920
catacgtat ttatttgctt ggtactgtt cttttgtcga tgetcaccct gttgtttggt 1980
gttacttctg cag 1993

```

```

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Zea maize
<220> FEATURE:
<221> NAME/KEY: signal
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: ER targeting sequence monocot

```

```

<400> SEQUENCE: 19

```

```

Met Arg Val Leu Leu Val Ala Leu Ala Leu Leu Ala Leu Ala Ala Ala
1          5          10          15

```

```

Ser Ala Thr Ser
          20

```

```

<210> SEQ ID NO 20
<211> LENGTH: 1698
<212> TYPE: DNA
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: artificial sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1698)
<223> OTHER INFORMATION: monocot optimized sucrose isomerase

```

```

<400> SEQUENCE: 20

```

```

gtg gcc gtg aac gac ggc gtg tcc gcc cac cca gtg tgg tgg aag gag 48
Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys Glu
1          5          10          15

```

```

gcc gtt ttc tac cag gtg tac ccg cgc agc ttc aag gac agc gac ggc 96
Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp Gly
          20          25          30

```

```

gac ggc atc ggc gac ctg aag ggc ctg acc gag aag ctg gac tac ctg 144
Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr Leu
          35          40          45

```

```

aag gcc ctg ggc atc aac gcc atc tgg atc aac ccg cac tac gac agc 192
Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp Ser
          50          55          60

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cgc aac acc gac aac ggc tac gat atc cgc gac tac cgc aag atc atg Pro Asn Thr Asp Asn Gly Tyr Asp Ile Arg Asp Tyr Arg Lys Ile Met 65 70 75 80	240
aag gaa tac ggc acg atg gac gac ttc gac cgc ctg atc gcc gag atg Lys Glu Tyr Gly Thr Met Asp Asp Phe Asp Arg Leu Ile Ala Glu Met 85 90 95	288
aag aag cgc gac atg cgc ctg atg atc gac gtg gtg gtg aac cac acc Lys Lys Arg Asp Met Arg Leu Met Ile Asp Val Val Val Asn His Thr 100 105 110	336
agc gac gag cac gag tgg ttc gtg gag agc aag aag tcc aag gac aac Ser Asp Glu His Glu Trp Phe Val Glu Ser Lys Lys Ser Lys Asp Asn 115 120 125	384
cgc tac cgc gac tac tac atc tgg cgc gac ggc aag gac ggc acc cag Pro Tyr Arg Asp Tyr Tyr Ile Trp Arg Asp Gly Lys Asp Gly Thr Gln 130 135 140	432
cgc aac aac tac cgc agc ttc ttc ggc ggc agc gcc tgg cag aag gac Pro Asn Asn Tyr Pro Ser Phe Phe Gly Gly Ser Ala Trp Gln Lys Asp 145 150 155 160	480
aac gcc acc cag cag tac tac ctg cac tac ttc ggc gtc cag cag ccg Asn Ala Thr Gln Gln Tyr Tyr Leu His Tyr Phe Gly Val Gln Gln Pro 165 170 175	528
gac ctg aac tgg gac aac ccg aaa gtg agg gag gag gtg tac gac atg Asp Leu Asn Trp Asp Asn Pro Lys Val Arg Glu Glu Val Tyr Asp Met 180 185 190	576
ctg agg ttc tgg atc gac aag ggc gtg tcc ggc ctg agg atg gac acc Leu Arg Phe Trp Ile Asp Lys Gly Val Ser Gly Leu Arg Met Asp Thr 195 200 205	624
gtg gcc acc ttc agc aag aac ccg gcc ttc ccg gac ctg acc ccg aag Val Ala Thr Phe Ser Lys Asn Pro Ala Phe Pro Asp Leu Thr Pro Lys 210 215 220	672
cag ctc cag aac ttc gcc tac acc tac acc cag ggc ccg aac ctg cac Gln Leu Gln Asn Phe Ala Tyr Thr Tyr Thr Gln Gly Pro Asn Leu His 225 230 235 240	720
cgc tac atc cag gag atg cac cag aag gtc ctg gcc aag tac gac gtg Arg Tyr Ile Gln Glu Met His Gln Lys Val Leu Ala Lys Tyr Asp Val 245 250 255	768
gtg tct gcc ggc gag atc ttc ggc gtg ccg ctc gag gag gcc gct ccg Val Ser Ala Gly Glu Ile Phe Gly Val Pro Leu Glu Glu Ala Ala Pro 260 265 270	816
ttc atc gac cag cgc ccg aag gaa ctg gac atg gcc ttc agc ttc gac Phe Ile Asp Gln Arg Arg Lys Glu Leu Asp Met Ala Phe Ser Phe Asp 275 280 285	864
ctg atc cgc ctc gac agg gcc gtg gag gag agg tgg cgc cgc aac gac Leu Ile Arg Leu Asp Arg Ala Val Glu Glu Arg Trp Arg Arg Asn Asp 290 295 300	912
tgg acc ctg agc cag ttc cgc cag atc aac aac cgc ctg gtg gac atg Trp Thr Leu Ser Gln Phe Arg Gln Ile Asn Asn Arg Leu Val Asp Met 305 310 315 320	960
gcc ggc cag cac ggc tgg aac acg ttc ttc ctc agc aac cac gac aac Ala Gly Gln His Trp Asn Thr Phe Phe Leu Ser Asn His Asp Asn 325 330 335	1008
ccg agg gcc gtg tcc cac ttc ggc gac gac agg cca gag tgg agg acc Pro Arg Ala Val Ser His Phe Gly Asp Asp Arg Pro Glu Trp Arg Thr 340 345 350	1056
cgc agc gcc aag gcc ctg gcc acc ctg gcc ctg acc cag agg gct acc Arg Ser Ala Lys Ala Leu Ala Thr Leu Ala Leu Thr Gln Arg Ala Thr 355 360 365	1104
cca ttc atc tac cag ggc gac gag ctg ggc atg acc aac tac ccg ttc Pro Phe Ile Tyr Gln Gly Asp Glu Leu Gly Met Thr Asn Tyr Pro Phe	1152

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370	375	380	
acc agc ctg agc gag ttc gac gat atc gag gtg aag ggc ttc tgg cag			1200
Thr Ser Leu Ser Glu Phe Asp Asp Ile Glu Val Lys Gly Phe Trp Gln			
385	390	395	400
gac ttc gtg gag act ggc aag gtg aag cca gac gtg ttc ctc gag aac			1248
Asp Phe Val Glu Thr Gly Lys Val Lys Pro Asp Val Phe Leu Glu Asn			
	405	410	415
gtg aag cag acc agc cgc gac aac agc cgc acc ccg ttc cag tgg agc			1296
Val Lys Gln Thr Ser Arg Asp Asn Ser Arg Thr Pro Phe Gln Trp Ser			
	420	425	430
aac acc gcc cag gcc ggc ttc acc acc ggc acc ccg tgg ttc cgc atc			1344
Asn Thr Ala Gln Ala Gly Phe Thr Thr Gly Thr Pro Trp Phe Arg Ile			
	435	440	445
aac ccg aac tac aag aac atc aac gcc gag gag cag acc cag aac ccg			1392
Asn Pro Asn Tyr Lys Asn Ile Asn Ala Glu Glu Gln Thr Gln Asn Pro			
	450	455	460
gac agc atc ttc cac ttc tac cgc cag ctg atc gag ctg agg cac gcc			1440
Asp Ser Ile Phe His Phe Tyr Arg Gln Leu Ile Glu Leu Arg His Ala			
	465	470	475
acc ccg gcc ttc acc tac ggc acc tac cag gac ctg gac ccg aac aac			1488
Thr Pro Ala Phe Thr Tyr Gly Thr Tyr Gln Asp Leu Asp Pro Asn Asn			
	485	490	495
aac gag gtg ctg gcc tac acc cgc gag ctg aac cag cag cgc tac ctg			1536
Asn Glu Val Leu Ala Tyr Thr Arg Glu Leu Asn Gln Gln Arg Tyr Leu			
	500	505	510
gtg gtg gtc aac ttc aag gag aag ccg gtc cac tac gtg ctg ccc aag			1584
Val Val Val Asn Phe Lys Glu Lys Pro Val His Tyr Val Leu Pro Lys			
	515	520	525
acc ctg agc atc aag cag agc ctg ctc gag agc ggc cag aag gac aag			1632
Thr Leu Ser Ile Lys Gln Ser Leu Leu Glu Ser Gly Gln Lys Asp Lys			
	530	535	540
gtc gag ccg aac gcc acc acc ctc gag ctt cag ccc tgg cag agc ggc			1680
Val Glu Pro Asn Ala Thr Thr Leu Glu Leu Gln Pro Trp Gln Ser Gly			
	545	550	555
atc tat cag ctg aac tga			1698
Ile Tyr Gln Leu Asn			
	565		

<210> SEQ ID NO 21

<211> LENGTH: 565

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 21

Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys Glu
1 5 10 15

Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp Gly
20 25 30

Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr Leu
35 40 45

Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp Ser
50 55 60

Pro Asn Thr Asp Asn Gly Tyr Asp Ile Arg Asp Tyr Arg Lys Ile Met
65 70 75 80

Lys Glu Tyr Gly Thr Met Asp Asp Phe Asp Arg Leu Ile Ala Glu Met
85 90 95

Lys Lys Arg Asp Met Arg Leu Met Ile Asp Val Val Val Asn His Thr

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100							105					110				
Ser	Asp	Glu	His	Glu	Trp	Phe	Val	Glu	Ser	Lys	Lys	Ser	Lys	Asp	Asn	
		115					120					125				
Pro	Tyr	Arg	Asp	Tyr	Tyr	Ile	Trp	Arg	Asp	Gly	Lys	Asp	Gly	Thr	Gln	
	130					135					140					
Pro	Asn	Asn	Tyr	Pro	Ser	Phe	Phe	Gly	Gly	Ser	Ala	Trp	Gln	Lys	Asp	
	145				150					155					160	
Asn	Ala	Thr	Gln	Gln	Tyr	Tyr	Leu	His	Tyr	Phe	Gly	Val	Gln	Gln	Pro	
			165						170					175		
Asp	Leu	Asn	Trp	Asp	Asn	Pro	Lys	Val	Arg	Glu	Glu	Val	Tyr	Asp	Met	
		180						185					190			
Leu	Arg	Phe	Trp	Ile	Asp	Lys	Gly	Val	Ser	Gly	Leu	Arg	Met	Asp	Thr	
		195					200					205				
Val	Ala	Thr	Phe	Ser	Lys	Asn	Pro	Ala	Phe	Pro	Asp	Leu	Thr	Pro	Lys	
	210					215					220					
Gln	Leu	Gln	Asn	Phe	Ala	Tyr	Thr	Tyr	Thr	Gln	Gly	Pro	Asn	Leu	His	
	225				230					235					240	
Arg	Tyr	Ile	Gln	Glu	Met	His	Gln	Lys	Val	Leu	Ala	Lys	Tyr	Asp	Val	
			245						250					255		
Val	Ser	Ala	Gly	Glu	Ile	Phe	Gly	Val	Pro	Leu	Glu	Glu	Ala	Ala	Pro	
			260					265					270			
Phe	Ile	Asp	Gln	Arg	Arg	Lys	Glu	Leu	Asp	Met	Ala	Phe	Ser	Phe	Asp	
		275					280					285				
Leu	Ile	Arg	Leu	Asp	Arg	Ala	Val	Glu	Glu	Arg	Trp	Arg	Arg	Asn	Asp	
	290					295					300					
Trp	Thr	Leu	Ser	Gln	Phe	Arg	Gln	Ile	Asn	Asn	Arg	Leu	Val	Asp	Met	
	305				310					315					320	
Ala	Gly	Gln	His	Gly	Trp	Asn	Thr	Phe	Phe	Leu	Ser	Asn	His	Asp	Asn	
			325					330						335		
Pro	Arg	Ala	Val	Ser	His	Phe	Gly	Asp	Asp	Arg	Pro	Glu	Trp	Arg	Thr	
		340						345					350			
Arg	Ser	Ala	Lys	Ala	Leu	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Arg	Ala	Thr	
		355					360					365				
Pro	Phe	Ile	Tyr	Gln	Gly	Asp	Glu	Leu	Gly	Met	Thr	Asn	Tyr	Pro	Phe	
	370				375						380					
Thr	Ser	Leu	Ser	Glu	Phe	Asp	Asp	Ile	Glu	Val	Lys	Gly	Phe	Trp	Gln	
	385				390					395					400	
Asp	Phe	Val	Glu	Thr	Gly	Lys	Val	Lys	Pro	Asp	Val	Phe	Leu	Glu	Asn	
			405						410					415		
Val	Lys	Gln	Thr	Ser	Arg	Asp	Asn	Ser	Arg	Thr	Pro	Phe	Gln	Trp	Ser	
		420						425					430			
Asn	Thr	Ala	Gln	Ala	Gly	Phe	Thr	Thr	Gly	Thr	Pro	Trp	Phe	Arg	Ile	
		435					440					445				
Asn	Pro	Asn	Tyr	Lys	Asn	Ile	Asn	Ala	Glu	Glu	Gln	Thr	Gln	Asn	Pro	
	450					455					460					
Asp	Ser	Ile	Phe	His	Phe	Tyr	Arg	Gln	Leu	Ile	Glu	Leu	Arg	His	Ala	
	465				470					475					480	
Thr	Pro	Ala	Phe	Thr	Tyr	Gly	Thr	Tyr	Gln	Asp	Leu	Asp	Pro	Asn	Asn	
			485						490					495		
Asn	Glu	Val	Leu	Ala	Tyr	Thr	Arg	Glu	Leu	Asn	Gln	Gln	Arg	Tyr	Leu	
		500						505					510			
Val	Val	Val	Asn	Phe	Lys	Glu	Lys	Pro	Val	His	Tyr	Val	Leu	Pro	Lys	
		515					520					525				

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Thr Leu Ser Ile Lys Gln Ser Leu Leu Glu Ser Gly Gln Lys Asp Lys
530 535 540

Val Glu Pro Asn Ala Thr Thr Leu Glu Leu Gln Pro Trp Gln Ser Gly
545 550 555 560

Ile Tyr Gln Leu Asn
565

<210> SEQ ID NO 22
<211> LENGTH: 194
<212> TYPE: DNA
<213> ORGANISM: Fig wort mosaic virus
<220> FEATURE:
<221> NAME/KEY: enhancer
<222> LOCATION: (1)..(194)

<400> SEQUENCE: 22

agctgcttgt ggggaccaga caaaaaagga atggtgcaga attgttaggc gcacctacca 60
aaagcatctt tgcctttatt gcaagataa agcagattcc tctagtacaa gtggggaaca 120
aaataacgtg gaaaagagct gtccctgacag cccactcact aatgcgtatg acgaacgcag 180
tgacgaccac aaaa 194

<210> SEQ ID NO 23
<211> LENGTH: 293
<212> TYPE: DNA
<213> ORGANISM: 35S virus
<220> FEATURE:
<221> NAME/KEY: enhancer
<222> LOCATION: (1)..(293)

<400> SEQUENCE: 23

acttttcaac aaagggtaat atccggaaac ctctctggat tccattgccc agtatctgt 60
cactttattg tgaagatagt ggaaaaggaa ggtggctcct acaaatgcca tcattgcgat 120
aaaggaaagg ctatcggtga agatgcctct gccgacagtg gtcccaaaga tggaccccca 180
cccacgagga gcatcggtga aaaagaagac gttccaacca cgtcttcaaa gcaagtggat 240
tgatgtgata tctccactga cgtaagggat gacgaacaat cccactatcc ttc 293

<210> SEQ ID NO 24
<211> LENGTH: 1701
<212> TYPE: DNA
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: artificial sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1701)
<223> OTHER INFORMATION: monocot optimized sucrose isomerase

<400> SEQUENCE: 24

atg gtg gcc gtg aac gac ggc gtg tcc gcc cac cca gtg tgg tgg aag 48
Met Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys
1 5 10 15
gag gcc gtt ttc tac cag gtg tac ccg cgc agc ttc aag gac agc gac 96
Glu Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp
20 25 30
ggc gac ggc atc ggc gac ctg aag ggc ctg acc gag aag ctg gac tac 144
Gly Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr
35 40 45
ctg aag gcc ctg ggc atc aac gcc atc tgg atc aac ccg cac tac gac 192
Leu Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp
50 55 60

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agc	ccg	aac	acc	gac	aac	ggc	tac	gat	atc	cgc	gac	tac	cgc	aag	atc	240
Ser	Pro	Asn	Thr	Asp	Asn	Gly	Tyr	Asp	Ile	Arg	Asp	Tyr	Arg	Lys	Ile	
65					70					75				80		
atg	aag	gaa	tac	ggc	acg	atg	gac	gac	ttc	gac	cgc	ctg	atc	gcc	gag	288
Met	Lys	Glu	Tyr	Gly	Thr	Met	Asp	Asp	Phe	Asp	Arg	Leu	Ile	Ala	Glu	
				85					90					95		
atg	aag	aag	cgc	gac	atg	cgc	ctg	atg	atc	gac	gtg	gtg	gtg	aac	cac	336
Met	Lys	Lys	Arg	Asp	Met	Arg	Leu	Met	Ile	Asp	Val	Val	Val	Asn	His	
			100					105					110			
acc	agc	gac	gag	cac	gag	tgg	ttc	gtg	gag	agc	aag	aag	tcc	aag	gac	384
Thr	Ser	Asp	Glu	His	Glu	Trp	Phe	Val	Glu	Ser	Lys	Lys	Ser	Lys	Asp	
			115			120							125			
aac	ccg	tac	cgc	gac	tac	tac	atc	tgg	cgc	gac	ggc	aag	gac	ggc	acc	432
Asn	Pro	Tyr	Arg	Asp	Tyr	Tyr	Ile	Trp	Arg	Asp	Gly	Lys	Asp	Gly	Thr	
	130					135					140					
cag	ccg	aac	aac	tac	ccg	agc	ttc	ttc	ggc	ggc	agc	gcc	tgg	cag	aag	480
Gln	Pro	Asn	Asn	Tyr	Pro	Ser	Phe	Phe	Gly	Gly	Ser	Ala	Trp	Gln	Lys	
	145				150					155				160		
gac	aac	gcc	acc	cag	cag	tac	tac	ctg	cac	tac	ttc	ggc	gtc	cag	cag	528
Asp	Asn	Ala	Thr	Gln	Gln	Tyr	Tyr	Leu	His	Tyr	Phe	Gly	Val	Gln	Gln	
				165					170					175		
ccg	gac	ctg	aac	tgg	gac	aac	ccg	aaa	gtg	agg	gag	gag	gtg	tac	gac	576
Pro	Asp	Leu	Asn	Trp	Asp	Asn	Pro	Lys	Val	Arg	Glu	Glu	Val	Tyr	Asp	
			180					185					190			
atg	ctg	agg	ttc	tgg	atc	gac	aag	ggc	gtg	tcc	ggc	ctg	agg	atg	gac	624
Met	Leu	Arg	Phe	Trp	Ile	Asp	Lys	Gly	Val	Ser	Gly	Leu	Arg	Met	Asp	
		195					200					205				
acc	gtg	gcc	acc	ttc	agc	aag	aac	ccg	gcc	ttc	ccg	gac	ctg	acc	ccg	672
Thr	Val	Ala	Thr	Phe	Ser	Lys	Asn	Pro	Ala	Phe	Pro	Asp	Leu	Thr	Pro	
	210					215					220					
aag	cag	ctc	cag	aac	ttc	gcc	tac	acc	tac	acc	cag	ggc	ccg	aac	ctg	720
Lys	Gln	Leu	Gln	Asn	Phe	Ala	Tyr	Thr	Tyr	Thr	Gln	Gly	Pro	Asn	Leu	
	225				230					235				240		
cac	cgc	tac	atc	cag	gag	atg	cac	cag	aag	gtc	ctg	gcc	aag	tac	gac	768
His	Arg	Tyr	Ile	Gln	Glu	Met	His	Gln	Lys	Val	Leu	Ala	Lys	Tyr	Asp	
				245					250					255		
gtg	gtg	tct	gcc	ggc	gag	atc	ttc	ggc	gtg	ccg	ctc	gag	gag	gcc	gct	816
Val	Val	Ser	Ala	Gly	Glu	Ile	Phe	Gly	Val	Pro	Leu	Glu	Glu	Ala	Ala	
			260					265						270		
ccg	ttc	atc	gac	cag	cgc	cgg	aag	gaa	ctg	gac	atg	gcc	ttc	agc	ttc	864
Pro	Phe	Ile	Asp	Gln	Arg	Arg	Lys	Glu	Leu	Asp	Met	Ala	Phe	Ser	Phe	
		275					280					285				
gac	ctg	atc	cgc	ctc	gac	agg	gcc	gtg	gag	gag	agg	tgg	cgc	cgc	aac	912
Asp	Leu	Ile	Arg	Leu	Asp	Arg	Ala	Val	Glu	Glu	Arg	Trp	Arg	Arg	Asn	
		290				295					300					
gac	tgg	acc	ctg	agc	cag	ttc	cgc	cag	atc	aac	aac	cgc	ctg	gtg	gac	960
Asp	Trp	Thr	Leu	Ser	Gln	Phe	Arg	Gln	Ile	Asn	Asn	Arg	Leu	Val	Asp	
	305				310				315					320		
atg	gcc	ggc	cag	cac	ggc	tgg	aac	acg	ttc	ttc	ctc	agc	aac	cac	gac	1008
Met	Ala	Gly	Gln	His	Gly	Trp	Asn	Thr	Phe	Phe	Leu	Ser	Asn	His	Asp	
			325					330						335		
aac	ccg	agg	gcc	gtg	tcc	cac	ttc	ggc	gac	gac	agg	cca	gag	tgg	agg	1056
Asn	Pro	Arg	Ala	Val	Ser	His	Phe	Gly	Asp	Asp	Arg	Pro	Glu	Trp	Arg	
			340					345					350			
acc	cgc	agc	gcc	aag	gcc	ctg	gcc	acc	ctg	gcc	ctg	acc	cag	agg	gct	1104
Thr	Arg	Ser	Ala	Lys	Ala	Leu	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Arg	Ala	
			355			360						365				
acc	cca	ttc	atc	tac	cag	ggc	gac	gag	ctg	ggc	atg	acc	aac	tac	ccg	1152
Thr	Pro	Phe	Ile	Tyr	Gln	Gly	Asp	Glu	Leu	Gly	Met	Thr	Asn	Tyr	Pro	

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370	375	380	
ttc acc agc ctg agc gag ttc gac gat atc gag gtg aag ggc ttc tgg			1200
Phe Thr Ser Leu Ser Glu Phe Asp Asp Ile Glu Val Lys Gly Phe Trp			
385	390	395	400
cag gac ttc gtg gag act ggc aag gtg aag cca gac gtg ttc ctc gag			1248
Gln Asp Phe Val Glu Thr Gly Lys Val Lys Pro Asp Val Phe Leu Glu			
	405	410	415
aac gtg aag cag acc agc cgc gac aac agc cgc acc ccg ttc cag tgg			1296
Asn Val Lys Gln Thr Ser Arg Asp Asn Ser Arg Thr Pro Phe Gln Trp			
	420	425	430
agc aac acc gcc cag gcc ggc ttc acc acc ggc acc ccg tgg ttc cgc			1344
Ser Asn Thr Ala Gln Ala Gly Phe Thr Thr Gly Thr Pro Trp Phe Arg			
	435	440	445
atc aac ccg aac tac aag aac atc aac gcc gag gag cag acc cag aac			1392
Ile Asn Pro Asn Tyr Lys Asn Ile Asn Ala Glu Glu Gln Thr Gln Asn			
	450	455	460
ccg gac agc atc ttc cac ttc tac cgc cag ctg atc gag ctg agg cac			1440
Pro Asp Ser Ile Phe His Phe Tyr Arg Gln Leu Ile Glu Leu Arg His			
	465	470	475
gcc acc ccg gcc ttc acc tac ggc acc tac cag gac ctg gac ccg aac			1488
Ala Thr Pro Ala Phe Thr Tyr Gly Thr Tyr Gln Asp Leu Asp Pro Asn			
	485	490	495
aac aac gag gtg ctg gcc tac acc cgc gag ctg aac cag cag cgc tac			1536
Asn Asn Glu Val Leu Ala Tyr Thr Arg Glu Leu Asn Gln Gln Arg Tyr			
	500	505	510
ctg gtg gtg gtc aac ttc aag gag aag ccg gtc cac tac gtg ctg ccc			1584
Leu Val Val Val Asn Phe Lys Glu Lys Pro Val His Tyr Val Leu Pro			
	515	520	525
aag acc ctg agc atc aag cag agc ctg ctc gag agc ggc cag aag gac			1632
Lys Thr Leu Ser Ile Lys Gln Ser Leu Leu Glu Ser Gly Gln Lys Asp			
	530	535	540
aag gtc gag ccg aac gcc acc acc ctc gag ctt cag ccc tgg cag agc			1680
Lys Val Glu Pro Asn Ala Thr Thr Leu Glu Leu Gln Pro Trp Gln Ser			
	545	550	555
ggc atc tat cag ctg aac tga			1701
Gly Ile Tyr Gln Leu Asn			
	565		

<210> SEQ ID NO 25

<211> LENGTH: 566

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 25

Met Val Ala Val Asn Asp Gly Val Ser Ala His Pro Val Trp Trp Lys	
1	5
Glu Ala Val Phe Tyr Gln Val Tyr Pro Arg Ser Phe Lys Asp Ser Asp	
	20
Gly Asp Gly Ile Gly Asp Leu Lys Gly Leu Thr Glu Lys Leu Asp Tyr	
	35
Leu Lys Ala Leu Gly Ile Asn Ala Ile Trp Ile Asn Pro His Tyr Asp	
	50
Ser Pro Asn Thr Asp Asn Gly Tyr Asp Ile Arg Asp Tyr Arg Lys Ile	
65	70
Met Lys Glu Tyr Gly Thr Met Asp Asp Phe Asp Arg Leu Ile Ala Glu	
	85
Met Lys Lys Arg Asp Met Arg Leu Met Ile Asp Val Val Val Asn His	

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100							105					110				
Thr	Ser	Asp	Glu	His	Glu	Trp	Phe	Val	Glu	Ser	Lys	Lys	Ser	Lys	Asp	
		115					120					125				
Asn	Pro	Tyr	Arg	Asp	Tyr	Tyr	Ile	Trp	Arg	Asp	Gly	Lys	Asp	Gly	Thr	
		130			135						140					
Gln	Pro	Asn	Asn	Tyr	Pro	Ser	Phe	Phe	Gly	Gly	Ser	Ala	Trp	Gln	Lys	
		145			150				155						160	
Asp	Asn	Ala	Thr	Gln	Gln	Tyr	Tyr	Leu	His	Tyr	Phe	Gly	Val	Gln	Gln	
				165				170						175		
Pro	Asp	Leu	Asn	Trp	Asp	Asn	Pro	Lys	Val	Arg	Glu	Glu	Val	Tyr	Asp	
		180						185				190				
Met	Leu	Arg	Phe	Trp	Ile	Asp	Lys	Gly	Val	Ser	Gly	Leu	Arg	Met	Asp	
		195				200						205				
Thr	Val	Ala	Thr	Phe	Ser	Lys	Asn	Pro	Ala	Phe	Pro	Asp	Leu	Thr	Pro	
		210				215				220						
Lys	Gln	Leu	Gln	Asn	Phe	Ala	Tyr	Thr	Tyr	Thr	Gln	Gly	Pro	Asn	Leu	
		225				230				235				240		
His	Arg	Tyr	Ile	Gln	Glu	Met	His	Gln	Lys	Val	Leu	Ala	Lys	Tyr	Asp	
				245						250				255		
Val	Val	Ser	Ala	Gly	Glu	Ile	Phe	Gly	Val	Pro	Leu	Glu	Glu	Ala	Ala	
		260						265				270				
Pro	Phe	Ile	Asp	Gln	Arg	Arg	Lys	Glu	Leu	Asp	Met	Ala	Phe	Ser	Phe	
		275				280						285				
Asp	Leu	Ile	Arg	Leu	Asp	Arg	Ala	Val	Glu	Glu	Arg	Trp	Arg	Arg	Asn	
		290				295						300				
Asp	Trp	Thr	Leu	Ser	Gln	Phe	Arg	Gln	Ile	Asn	Asn	Arg	Leu	Val	Asp	
		305				310				315				320		
Met	Ala	Gly	Gln	His	Gly	Trp	Asn	Thr	Phe	Phe	Leu	Ser	Asn	His	Asp	
				325						330				335		
Asn	Pro	Arg	Ala	Val	Ser	His	Phe	Gly	Asp	Asp	Arg	Pro	Glu	Trp	Arg	
		340						345				350				
Thr	Arg	Ser	Ala	Lys	Ala	Leu	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Arg	Ala	
		355				360						365				
Thr	Pro	Phe	Ile	Tyr	Gln	Gly	Asp	Glu	Leu	Gly	Met	Thr	Asn	Tyr	Pro	
		370				375						380				
Phe	Thr	Ser	Leu	Ser	Glu	Phe	Asp	Asp	Ile	Glu	Val	Lys	Gly	Phe	Trp	
		385				390				395				400		
Gln	Asp	Phe	Val	Glu	Thr	Gly	Lys	Val	Lys	Pro	Asp	Val	Phe	Leu	Glu	
				405						410				415		
Asn	Val	Lys	Gln	Thr	Ser	Arg	Asp	Asn	Ser	Arg	Thr	Pro	Phe	Gln	Trp	
		420						425				430				
Ser	Asn	Thr	Ala	Gln	Ala	Gly	Phe	Thr	Thr	Gly	Thr	Pro	Trp	Phe	Arg	
		435				440						445				
Ile	Asn	Pro	Asn	Tyr	Lys	Asn	Ile	Asn	Ala	Glu	Glu	Gln	Thr	Gln	Asn	
		450				455						460				
Pro	Asp	Ser	Ile	Phe	His	Phe	Tyr	Arg	Gln	Leu	Ile	Glu	Leu	Arg	His	
		465				470				475				480		
Ala	Thr	Pro	Ala	Phe	Thr	Tyr	Gly	Thr	Tyr	Gln	Asp	Leu	Asp	Pro	Asn	
				485						490				495		
Asn	Asn	Glu	Val	Leu	Ala	Tyr	Thr	Arg	Glu	Leu	Asn	Gln	Gln	Arg	Tyr	
		500						505				510				
Leu	Val	Val	Val	Asn	Phe	Lys	Glu	Lys	Pro	Val	His	Tyr	Val	Leu	Pro	
		515				520						525				

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Lys Thr Leu Ser Ile Lys Gln Ser Leu Leu Glu Ser Gly Gln Lys Asp
530 535 540

Lys Val Glu Pro Asn Ala Thr Thr Leu Glu Leu Gln Pro Trp Gln Ser
545 550 555 560

Gly Ile Tyr Gln Leu Asn
565

<210> SEQ ID NO 26
<211> LENGTH: 65
<212> TYPE: PRT
<213> ORGANISM: *Cyanophora paradoxa*
<220> FEATURE:
<221> NAME/KEY: signal
<222> LOCATION: (1)..(65)
<223> OTHER INFORMATION: FNR plastid targeting sequence

<400> SEQUENCE: 26

Met Ala Phe Val Ala Ser Val Pro Val Phe Ala Asn Ala Ser Gly Leu
1 5 10 15

Lys Thr Glu Ala Lys Val Cys Gln Lys Pro Ala Leu Lys Asn Ser Phe
20 25 30

Phe Arg Gly Glu Glu Val Thr Ser Arg Ser Phe Phe Ala Ser Gln Ala
35 40 45

Val Ser Ala Lys Pro Ala Thr Thr Gly Glu Val Asp Thr Thr Ile Arg
50 55 60

Ala
65

<210> SEQ ID NO 27
<211> LENGTH: 1764
<212> TYPE: DNA
<213> ORGANISM: *Bacillus*
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1764)
<223> OTHER INFORMATION: dicot optimized alpha-1,1-glucosidase

<400> SEQUENCE: 27

atg tcc act gct ctt act cag act tct act aac tct cag cag tct cca 48
Met Ser Thr Ala Leu Thr Gln Thr Ser Thr Asn Ser Gln Gln Ser Pro
1 5 10 15

att aga agg gct tgg tgg aaa gag gct gtt gtt tac caa atc tac cca 96
Ile Arg Arg Ala Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro
20 25 30

cgt tct ttc atg gat tcc aac ggt gat gga att gga gat ctt agg gga 144
Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Ile Gly Asp Leu Arg Gly
35 40 45

att ctc tcc aag ttg gat tac ctt aag ttg ctc gga gtt gat gtt ctt 192
Ile Leu Ser Lys Leu Asp Tyr Leu Lys Leu Leu Gly Val Asp Val Leu
50 55 60

tgg ctc aac cca atc tac gat tcc cca aac gat gat atg gga tac gat 240
Trp Leu Asn Pro Ile Tyr Asp Ser Pro Asn Asp Asp Met Gly Tyr Asp
65 70 75 80

atc agg gat tac tac aag atc atg gaa gag ttc gga act atg gaa gat 288
Ile Arg Asp Tyr Tyr Lys Ile Met Glu Glu Phe Gly Thr Met Glu Asp
85 90 95

ttc gag gaa ctt ctt aga gaa gtt cac gct cgt gga atg aag ttg gtg 336
Phe Glu Glu Leu Leu Arg Glu Val His Ala Arg Gly Met Lys Leu Val
100 105 110

atg gat ctt gtt gct aac cac act tct gat gag cac cct tgg ttt att 384
Met Asp Leu Val Ala Asn His Thr Ser Asp Glu His Pro Trp Phe Ile

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115	120	125	
gag tct agg tcc tct agg gat aat cca tac cgt gat tgg tac att tgg Glu Ser Arg Ser Ser Arg Asp Asn Pro Tyr Arg Asp Trp Tyr Ile Trp 130 135 140			432
cgt gat cca aag gat gga aga gag cca aat aac tgg ctt tct tac ttc Arg Asp Pro Lys Asp Gly Arg Glu Pro Asn Asn Trp Leu Ser Tyr Phe 145 150 155 160			480
tct gga tct gct tgg gaa tat gat gag agg act gga cag tac tac ctt Ser Gly Ser Ala Trp Glu Tyr Asp Glu Arg Thr Gly Gln Tyr Tyr Leu 165 170 175			528
cac ttg ttc tct aga agg cag cca gat ctt aat tgg gag aac cca aaa His Leu Phe Ser Arg Arg Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys 180 185 190			576
gtg cgt gaa gct atc ttt gag atg atg agg ttc tgg ctc gat aag gga Val Arg Glu Ala Ile Phe Glu Met Met Arg Phe Trp Leu Asp Lys Gly 195 200 205			624
att gat gga ttc agg atg gat gtg atc aac gct att gct aag gct gaa Ile Asp Gly Phe Arg Met Asp Val Ile Asn Ala Ile Ala Lys Ala Glu 210 215 220			672
gga ctt cca gat gct cca gct aga cca ggt gaa aga tat gct tgg gga Gly Leu Pro Asp Ala Pro Ala Arg Pro Gly Glu Arg Tyr Ala Trp Gly 225 230 235 240			720
gga cag tat ttc ctt aac cag cca aag gtt cac gaa tac ctc aga gag Gly Gln Tyr Phe Leu Asn Gln Pro Lys Val His Glu Tyr Leu Arg Glu 245 250 255			768
atg tac gat aag gtt ctc tcc cac tac gat att atg act gtg gga gag Met Tyr Asp Lys Val Leu Ser His Tyr Asp Ile Met Thr Val Gly Glu 260 265 270			816
act ggt gga gtt act act aag gat gca ctc ttg ttc gct ggt gaa gat Thr Gly Gly Val Thr Thr Lys Asp Ala Leu Leu Phe Ala Gly Glu Asp 275 280 285			864
aga agg gaa ctc aac atg gtt ttc cag ttc gag cac atg gat atc gat Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Ile Asp 290 295 300			912
gct act gat ggt gat aag tgg agg cca aga cct tgg aga ctt act gag Ala Thr Asp Gly Asp Lys Trp Arg Pro Arg Pro Trp Arg Leu Thr Glu 305 310 315 320			960
ctt aag act atc atg act agg tgg cag aat gat ctt tat gga aag gct Leu Lys Thr Ile Met Thr Arg Trp Gln Asn Asp Leu Tyr Gly Lys Ala 325 330 335			1008
tgg aac tct ctc tac tgg act aat cat gat cag cca agg gct gtt tct Trp Asn Ser Leu Tyr Trp Thr Asn His Asp Gln Pro Arg Ala Val Ser 340 345 350			1056
aga ttc gga aac gat gga cca tat cgt gtt gag tct gct aag atg ctt Arg Phe Gly Asn Asp Gly Pro Tyr Arg Val Glu Ser Ala Lys Met Leu 355 360 365			1104
gct act gtg ctt cat atg atg caa ggt aca cct tac atc tac cag ggt Ala Thr Val Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly 370 375 380			1152
gaa gag att gga atg act aac tgc cca ttc gat tcc att gat gag tac Glu Glu Ile Gly Met Thr Asn Cys Pro Phe Asp Ser Ile Asp Glu Tyr 385 390 395 400			1200
cgt gat gtg gag att cat aac ctt tgg agg cac aga gtt atg gaa ggt Arg Asp Val Glu Ile His Asn Leu Trp Arg His Arg Val Met Glu Gly 405 410 415			1248
gga caa gat cca gct gaa gtt ctt agg gtg atc caa ctt aag gga agg Gly Gln Asp Pro Ala Glu Val Leu Arg Val Ile Gln Leu Lys Gly Arg 420 425 430			1296
gat aat gct aga act cca atg caa tgg gat gat tct cca aac gct gga			1344

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Asp	Asn	Ala	Arg	Thr	Pro	Met	Gln	Trp	Asp	Asp	Ser	Pro	Asn	Ala	Gly		
		435					440					445					
ttc	act	act	gga	aca	cct	tgg	att	aag	gtg	aac	cca	aac	tac	cga	gag		1392
Phe	Thr	Thr	Gly	Thr	Pro	Trp	Ile	Lys	Val	Asn	Pro	Asn	Tyr	Arg	Glu		
		450				455					460						
atc	aac	gtt	aag	cag	gct	ctt	gct	gat	cca	aac	tcc	atc	ttc	cat	tac		1440
Ile	Asn	Val	Lys	Gln	Ala	Leu	Ala	Asp	Pro	Asn	Ser	Ile	Phe	His	Tyr		
465					470					475					480		
tac	cgt	aga	ctt	atc	caa	ctt	agg	aag	cag	cat	cca	atc	gtt	gtt	tac		1488
Tyr	Arg	Arg	Leu	Ile	Gln	Leu	Arg	Lys	Gln	His	Pro	Ile	Val	Val	Tyr		
				485					490					495			
gga	aag	tac	gat	ctc	att	ctc	cca	gat	cac	gaa	gag	att	tgg	gct	tac		1536
Gly	Lys	Tyr	Asp	Leu	Ile	Leu	Pro	Asp	His	Glu	Glu	Ile	Trp	Ala	Tyr		
			500					505					510				
act	agg	act	ctt	gga	gat	gag	aga	tgg	ctt	atc	gtg	gct	aat	ttc	ttc		1584
Thr	Arg	Thr	Leu	Gly	Asp	Glu	Arg	Trp	Leu	Ile	Val	Ala	Asn	Phe	Phe		
		515				520						525					
gga	gga	act	cca	gaa	ttt	gaa	ctt	cca	cct	gaa	gtt	aga	tgt	gag	ggt		1632
Gly	Gly	Thr	Pro	Glu	Phe	Glu	Leu	Pro	Pro	Glu	Val	Arg	Cys	Glu	Gly		
		530				535					540						
gct	gag	ttg	gtt	att	gct	aac	tac	cca	gtg	gat	gat	tct	gaa	gca	ggt		1680
Ala	Glu	Leu	Val	Ile	Ala	Asn	Tyr	Pro	Val	Asp	Asp	Ser	Glu	Ala	Gly		
545					550					555					560		
gga	cca	gct	gct	gcc	ggt	gct	cct	cat	agg	ttt	agg	ctt	agg	cca	tat		1728
Gly	Pro	Ala	Ala	Ala	Gly	Ala	Pro	His	Arg	Phe	Arg	Leu	Arg	Pro	Tyr		
			565					570						575			
gag	tgt	cgt	gtt	tac	cgt	ctt	ttg	gga	tgg	cac	taa						1764
Glu	Cys	Arg	Val	Tyr	Arg	Leu	Leu	Gly	Trp	His							
			580					585									

<210> SEQ ID NO 28

<211> LENGTH: 587

<212> TYPE: PRT

<213> ORGANISM: Bacillus

<400> SEQUENCE: 28

Met	Ser	Thr	Ala	Leu	Thr	Gln	Thr	Ser	Thr	Asn	Ser	Gln	Gln	Ser	Pro		
1				5					10					15			
Ile	Arg	Arg	Ala	Trp	Trp	Lys	Glu	Ala	Val	Val	Tyr	Gln	Ile	Tyr	Pro		
			20			25							30				
Arg	Ser	Phe	Met	Asp	Ser	Asn	Gly	Asp	Gly	Ile	Gly	Asp	Leu	Arg	Gly		
		35				40						45					
Ile	Leu	Ser	Lys	Leu	Asp	Tyr	Leu	Lys	Leu	Leu	Gly	Val	Asp	Val	Leu		
	50				55						60						
Trp	Leu	Asn	Pro	Ile	Tyr	Asp	Ser	Pro	Asn	Asp	Asp	Met	Gly	Tyr	Asp		
65				70					75					80			
Ile	Arg	Asp	Tyr	Tyr	Lys	Ile	Met	Glu	Glu	Phe	Gly	Thr	Met	Glu	Asp		
			85					90						95			
Phe	Glu	Glu	Leu	Leu	Arg	Glu	Val	His	Ala	Arg	Gly	Met	Lys	Leu	Val		
			100					105						110			
Met	Asp	Leu	Val	Ala	Asn	His	Thr	Ser	Asp	Glu	His	Pro	Trp	Phe	Ile		
		115				120						125					
Glu	Ser	Arg	Ser	Ser	Arg	Asp	Asn	Pro	Tyr	Arg	Asp	Trp	Tyr	Ile	Trp		
		130				135						140					
Arg	Asp	Pro	Lys	Asp	Gly	Arg	Glu	Pro	Asn	Asn	Trp	Leu	Ser	Tyr	Phe		
				150						155					160		
Ser	Gly	Ser	Ala	Trp	Glu	Tyr	Asp	Glu	Arg	Thr	Gly	Gln	Tyr	Tyr	Leu		
			165					170						175			

His	Leu	Phe	Ser	Arg	Arg	Gln	Pro	Asp	Leu	Asn	Trp	Glu	Asn	Pro	Lys
			180					185					190		
Val	Arg	Glu	Ala	Ile	Phe	Glu	Met	Met	Arg	Phe	Trp	Leu	Asp	Lys	Gly
		195					200					205			
Ile	Asp	Gly	Phe	Arg	Met	Asp	Val	Ile	Asn	Ala	Ile	Ala	Lys	Ala	Glu
	210					215					220				
Gly	Leu	Pro	Asp	Ala	Pro	Ala	Arg	Pro	Gly	Glu	Arg	Tyr	Ala	Trp	Gly
225					230					235					240
Gly	Gln	Tyr	Phe	Leu	Asn	Gln	Pro	Lys	Val	His	Glu	Tyr	Leu	Arg	Glu
				245					250					255	
Met	Tyr	Asp	Lys	Val	Leu	Ser	His	Tyr	Asp	Ile	Met	Thr	Val	Gly	Glu
			260					265					270		
Thr	Gly	Gly	Val	Thr	Thr	Lys	Asp	Ala	Leu	Leu	Phe	Ala	Gly	Glu	Asp
		275					280					285			
Arg	Arg	Glu	Leu	Asn	Met	Val	Phe	Gln	Phe	Glu	His	Met	Asp	Ile	Asp
	290					295					300				
Ala	Thr	Asp	Gly	Asp	Lys	Trp	Arg	Pro	Arg	Pro	Trp	Arg	Leu	Thr	Glu
305					310					315					320
Leu	Lys	Thr	Ile	Met	Thr	Arg	Trp	Gln	Asn	Asp	Leu	Tyr	Gly	Lys	Ala
				325					330					335	
Trp	Asn	Ser	Leu	Tyr	Trp	Thr	Asn	His	Asp	Gln	Pro	Arg	Ala	Val	Ser
			340					345					350		
Arg	Phe	Gly	Asn	Asp	Gly	Pro	Tyr	Arg	Val	Glu	Ser	Ala	Lys	Met	Leu
	355					360						365			
Ala	Thr	Val	Leu	His	Met	Met	Gln	Gly	Thr	Pro	Tyr	Ile	Tyr	Gln	Gly
	370					375					380				
Glu	Glu	Ile	Gly	Met	Thr	Asn	Cys	Pro	Phe	Asp	Ser	Ile	Asp	Glu	Tyr
385					390					395					400
Arg	Asp	Val	Glu	Ile	His	Asn	Leu	Trp	Arg	His	Arg	Val	Met	Glu	Gly
				405					410					415	
Gly	Gln	Asp	Pro	Ala	Glu	Val	Leu	Arg	Val	Ile	Gln	Leu	Lys	Gly	Arg
			420					425					430		
Asp	Asn	Ala	Arg	Thr	Pro	Met	Gln	Trp	Asp	Asp	Ser	Pro	Asn	Ala	Gly
	435						440					445			
Phe	Thr	Thr	Gly	Thr	Pro	Trp	Ile	Lys	Val	Asn	Pro	Asn	Tyr	Arg	Glu
	450					455					460				
Ile	Asn	Val	Lys	Gln	Ala	Leu	Ala	Asp	Pro	Asn	Ser	Ile	Phe	His	Tyr
465				470						475					480
Tyr	Arg	Arg	Leu	Ile	Gln	Leu	Arg	Lys	Gln	His	Pro	Ile	Val	Val	Tyr
			485						490					495	
Gly	Lys	Tyr	Asp	Leu	Ile	Leu	Pro	Asp	His	Glu	Glu	Ile	Trp	Ala	Tyr
			500					505					510		
Thr	Arg	Thr	Leu	Gly	Asp	Glu	Arg	Trp	Leu	Ile	Val	Ala	Asn	Phe	Phe
		515					520					525			
Gly	Gly														

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<210> SEQ ID NO 29
 <211> LENGTH: 1541
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic gene
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(1541)
 <223> OTHER INFORMATION: dextranucrase with leucrose synthase activity

<400> SEQUENCE: 29

```

Met Leu Glu Ser Gly Val Val His Ala Asp Asp Val Lys Gln Val Val
1          5          10          15

Val Gln Glu Pro Ala Thr Ala Gln Thr Ser Gly Pro Gly Gln Gln Thr
20          25          30

Pro Ala Gln Ala Lys Ile Ala Ser Glu Gln Glu Ala Glu Lys Val Thr
35          40          45

Pro Ala Asp Lys Val Thr Asp Asp Val Ala Ala Ser Glu Lys Pro Ala
50          55          60

Lys Pro Ala Glu Asn Thr Glu Ala Thr Val Gln Thr Asn Ala Gln Glu
65          70          75          80

Pro Ala Lys Pro Ala Asp Thr Lys Glu Ala Ser Thr Glu Lys Ala Ala
85          90          95

Val Ala Glu Glu Val Lys Ala Ala Asn Ala Ile Thr Glu Ile Pro Lys
100         105         110

Thr Glu Val Ala Asp Gln Asn Lys Gln Ala Arg Pro Thr Thr Ala Gln
115         120         125

Asp Gln Glu Gly Asp Lys Arg Glu Lys Thr Ala Val Glu Asp Lys Ile
130         135         140

Val Ala Asn Pro Lys Val Ala Lys Lys Asp Arg Leu Pro Glu Pro Gly
145         150         155         160

Ser Lys Gln Gly Ala Ile Ala Glu Arg Met Val Ala Asp Gln Ala Gln
165         170         175

Pro Ala Pro Val Asn Ala Asp His Asp Asp Asp Val Leu Ser His Ile
180         185         190

Lys Thr Ile Asp Gly Lys Asn Tyr Tyr Val Gln Asp Asp Gly Thr Val
195         200         205

Lys Lys Asn Phe Ala Val Glu Leu Asn Gly Arg Ile Leu Tyr Phe Asp
210         215         220

Ala Glu Thr Gly Ala Leu Val Asp Ser Asn Glu Tyr Gln Phe Gln Gln
225         230         235         240

Gly Thr Ser Ser Leu Asn Asn Glu Phe Ser Gln Lys Asn Ala Phe Tyr
245         250         255

Gly Thr Thr Asp Lys Asp Ile Glu Thr Val Asp Gly Tyr Leu Thr Ala
260         265         270

Asp Ser Trp Tyr Arg Pro Lys Phe Ile Leu Lys Asp Gly Lys Thr Trp
275         280         285

Thr Ala Ser Thr Glu Thr Asp Leu Arg Pro Leu Leu Met Ala Trp Trp
290         295         300

Pro Asp Lys Arg Thr Gln Ile Asn Tyr Leu Asn Tyr Met Asn Gln Gln
305         310         315         320

Gly Leu Gly Ala Gly Ala Phe Glu Asn Lys Val Glu Gln Ala Leu Leu
325         330         335

Thr Gly Ala Ser Gln Gln Val Gln Arg Lys Ile Glu Glu Lys Ile Gly
340         345         350
  
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Lys	Glu	Gly	Asp	Thr	Lys	Trp	Leu	Arg	Thr	Leu	Met	Gly	Ala	Phe	Val	
		355														
Lys	Thr	Gln	Pro	Asn	Trp	Asn	Ile	Lys	Thr	Glu	Ser	Glu	Thr	Thr	Gly	
		370														
Thr	Lys	Lys	Asp	His	Leu	Gln	Gly	Gly	Ala	Leu	Leu	Tyr	Thr	Asn	Asn	
		385					390					395				
Glu	Lys	Ser	Pro	His	Ala	Asp	Ser	Lys	Phe	Arg	Leu	Leu	Asn	Arg	Thr	
						405					410					
Pro	Thr	Ser	Gln	Thr	Gly	Thr	Pro	Lys	Tyr	Phe	Ile	Asp	Lys	Ser	Asn	
						420					425					
Gly	Gly	Tyr	Glu	Phe	Leu	Leu	Ala	Asn	Asp	Phe	Asp	Asn	Ser	Asn	Pro	
						435					440					
Ala	Val	Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Tyr	Met	Met	Asn	Phe	
						450					455					
Gly	Ser	Ile	Val	Ala	Asn	Asp	Pro	Thr	Ala	Asn	Phe	Asp	Gly	Val	Arg	
						465					470					
Val	Asp	Ala	Val	Asp	Asn	Val	Asn	Ala	Asp	Leu	Leu	Gln	Ile	Ala	Ser	
						485					490					
Asp	Tyr	Phe	Lys	Ser	Arg	Tyr	Lys	Val	Gly	Glu	Ser	Glu	Glu	Glu	Ala	
						500					505					
Ile	Lys	His	Leu	Ser	Ile	Leu	Glu	Ala	Trp	Ser	Asp	Asn	Asp	Pro	Asp	
						515					520					
Tyr	Asn	Lys	Asp	Thr	Lys	Gly	Ala	Gln	Leu	Ala	Ile	Asp	Asn	Lys	Leu	
						530					535					
Arg	Leu	Ser	Leu	Leu	Tyr	Ser	Phe	Met	Arg	Asn	Leu	Ser	Ile	Arg	Ser	
						545					550					
Gly	Val	Glu	Pro	Thr	Ile	Thr	Asn	Ser	Leu	Asn	Asp	Arg	Ser	Ser	Glu	
						565					570					
Lys	Lys	Asn	Gly	Glu	Arg	Met	Ala	Asn	Tyr	Ile	Phe	Val	Arg	Ala	His	
						580					585					
Asp	Asp	Glu	Val	Gln	Thr	Val	Ile	Ala	Asp	Ile	Ile	Arg	Glu	Asn	Ile	
						595					600					
Asn	Pro	Asn	Thr	Asp	Gly	Leu	Thr	Phe	Thr	Met	Asp	Glu	Leu	Lys	Gln	
						610					615					
Ala	Phe	Lys	Ile	Tyr	Asn	Glu	Asp	Met	Arg	Lys	Ala	Asp	Lys	Lys	Tyr	
						625					630					
Thr	Gln	Phe	Asn	Ile	Pro	Thr	Ala	His	Ala	Leu	Met	Leu	Ser	Asn	Lys	
						645					650					
Asp	Ser	Ile	Thr	Arg	Val	Tyr	Tyr	Gly	Asp	Leu	Tyr	Thr	Asp	Asp	Gly	
						660					665					
Gln	Tyr	Met	Glu	Lys	Lys	Ser	Pro	Tyr	His	Asp	Ala	Ile	Asp	Ala	Leu	
						675					680					
Leu	Arg	Ala	Arg	Ile	Lys	Tyr	Val	Ala	Gly	Gly	Gln	Asp	Met	Lys	Val	
						690					695					
Thr	Tyr	Met	Gly	Val	Pro	Arg	Glu	Ala	Asp	Lys	Trp	Ser	Tyr	Asn	Gly	
						705					710					
Ile	Leu	Thr	Ser	Val	Arg	Tyr	Gly	Thr	Gly	Ala	Asn	Glu	Ala	Thr	Asp	
						725					730					
Glu	Gly	Thr	Ala	Glu	Thr	Arg	Thr	Gln	Gly	Met	Ala	Val	Ile	Ala	Ser	
						740					745					
Asn	Asn	Pro	Asn	Leu	Lys	Leu	Asn	Glu	Trp	Asp	Lys	Leu	Gln	Val	Asn	
						755					760					

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Met Gly Ala Ala His Lys Asn Gln Tyr Tyr Arg Pro Val Leu Leu Thr	770	775	780
Thr Lys Asp Gly Ile Ser Arg Tyr Leu Thr Asp Glu Glu Val Pro Gln	785	790	800
Ser Leu Trp Lys Lys Thr Asp Ala Asn Gly Ile Leu Thr Phe Asp Met	805	810	815
Asn Asp Ile Ala Gly Tyr Ser Asn Val Gln Val Ser Gly Tyr Leu Ala	820	825	830
Val Trp Val Pro Val Gly Ala Lys Ala Asp Gln Asp Ala Arg Thr Thr	835	840	845
Ala Ser Lys Lys Lys Asn Ala Ser Gly Gln Val Tyr Glu Ser Ser Ala	850	855	860
Ala Leu Asp Ser Gln Leu Ile Tyr Glu Gly Phe Ser Asn Phe Gln Asp	865	870	880
Phe Ala Thr Arg Asp Asp Gln Tyr Thr Asn Lys Val Ile Ala Lys Asn	885	890	895
Val Asn Leu Phe Lys Glu Trp Gly Val Thr Ser Phe Glu Leu Pro Pro	900	905	910
Gln Tyr Val Ser Ser Gln Asp Gly Thr Phe Leu Asp Ser Ile Ile Gln	915	920	925
Asn Gly Tyr Ala Phe Glu Asp Arg Tyr Asp Met Ala Met Ser Lys Asn	930	935	940
Asn Lys Tyr Gly Ser Leu Lys Asp Leu Leu Asn Ala Leu Arg Ala Leu	945	950	955
His Ser Val Asn Ile Gln Ala Ile Ala Asp Trp Val Pro Asp Gln Ile	965	970	975
Tyr Asn Leu Pro Gly Lys Glu Val Val Thr Ala Thr Arg Val Asn Asn	980	985	990
Tyr Gly Thr Tyr Arg Glu Gly Ala Glu Ile Lys Glu Lys Leu Tyr Val	995	1000	1005
Ala Asn Ser Lys Thr Asn Glu Thr Asp Phe Gln Gly Lys Tyr Gly	1010	1015	1020
Gly Ala Phe Leu Asp Glu Leu Lys Ala Lys Tyr Pro Glu Ile Phe	1025	1030	1035
Glu Arg Val Gln Ile Ser Asn Gly Gln Lys Met Thr Thr Asp Glu	1040	1045	1050
Lys Ile Thr Lys Trp Ser Ala Lys Tyr Phe Asn Gly Thr Asn Ile	1055	1060	1065
Leu Gly Arg Gly Ala Tyr Tyr Val Leu Lys Asp Trp Ala Ser Asn	1070	1075	1080
Asp Tyr Leu Thr Asn Arg Asn Gly Glu Ile Val Leu Pro Lys Gln	1085	1090	1095
Leu Val Asn Lys Asn Ser Tyr Thr Gly Phe Val Ser Asp Ala Asn	1100	1105	1110
Gly Thr Lys Phe Tyr Ser Thr Ser Gly Tyr Gln Ala Lys Asn Ser	1115	1120	1125
Phe Ile Gln Asp Glu Asn Gly Asn Trp Tyr Tyr Phe Asp Lys Arg	1130	1135	1140
Gly Tyr Leu Val Thr Gly Ala His Glu Ile Asp Gly Lys His Val	1145	1150	1155
Tyr Phe Leu Lys Asn Gly Ile Gln Leu Arg Asp Ser Ile Arg Glu	1160	1165	1170
Asp Glu Asn Gly Asn Gln Tyr Tyr Tyr Asp Gln Thr Gly Ala Gln			

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1175	1180	1185
Val Leu Asn Arg Tyr Tyr Thr Thr Asp Gly Gln Asn Trp Arg Tyr		
1190	1195	1200
Phe Asp Ala Lys Gly Val Met Ala Arg Gly Leu Val Lys Ile Gly		
1205	1210	1215
Asp Gly Gln Gln Phe Phe Asp Glu Asn Gly Tyr Gln Val Lys Gly		
1220	1225	1230
Lys Ile Val Ser Ala Lys Asp Gly Lys Leu Arg Tyr Phe Asp Lys		
1235	1240	1245
Asp Ser Gly Asn Ala Val Ile Asn Arg Phe Ala Gln Gly Asp Asn		
1250	1255	1260
Pro Ser Asp Trp Tyr Tyr Phe Gly Val Glu Phe Ala Lys Leu Thr		
1265	1270	1275
Gly Leu Gln Lys Ile Gly Gln Gln Thr Leu Tyr Phe Asp Gln Asp		
1280	1285	1290
Gly Lys Gln Val Lys Gly Lys Ile Val Thr Leu Ser Asp Lys Ser		
1295	1300	1305
Ile Arg Tyr Phe Asp Ala Asn Ser Gly Glu Met Ala Val Gly Lys		
1310	1315	1320
Phe Ala Glu Gly Ala Lys Asn Glu Trp Tyr Tyr Phe Asp Lys Thr		
1325	1330	1335
Gly Lys Ala Val Thr Gly Leu Gln Lys Ile Gly Lys Gln Thr Leu		
1340	1345	1350
Tyr Phe Asp Gln Asp Gly Lys Gln Val Lys Gly Lys Val Val Thr		
1355	1360	1365
Leu Ala Asp Lys Ser Ile Arg Tyr Phe Asp Ala Asp Ser Gly Glu		
1370	1375	1380
Met Ala Val Gly Lys Phe Ala Glu Gly Ala Lys Asn Glu Trp Tyr		
1385	1390	1395
Tyr Phe Asp Gln Thr Gly Lys Ala Val Thr Gly Leu Gln Lys Ile		
1400	1405	1410
Asp Lys Gln Thr Leu Tyr Phe Asp Gln Asp Gly Lys Gln Val Lys		
1415	1420	1425
Gly Lys Ile Val Thr Leu Ser Asp Lys Ser Ile Arg Tyr Phe Asp		
1430	1435	1440
Ala Asn Ser Gly Glu Met Ala Thr Asn Lys Phe Val Glu Gly Ser		
1445	1450	1455
Gln Asn Glu Trp Tyr Tyr Phe Asp Gln Ala Gly Lys Ala Val Thr		
1460	1465	1470
Gly Leu Gln Gln Val Gly Gln Gln Thr Leu Tyr Phe Thr Gln Asp		
1475	1480	1485
Gly Lys Gln Val Lys Gly Lys Val Val Asp Val Asn Gly Val Ser		
1490	1495	1500
Arg Tyr Phe Asp Ala Asn Ser Gly Asp Met Ala Arg Ser Lys Trp		
1505	1510	1515
Ile Gln Leu Glu Asp Gly Ser Trp Met Tyr Phe Asp Arg Asp Gly		
1520	1525	1530
Arg Gly Gln Asn Phe Gly Arg Asn		
1535	1540	

<210> SEQ ID NO 30

<211> LENGTH: 529

<212> TYPE: PRT

<213> ORGANISM: Thermus thermophilus

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<220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(508)
 <223> OTHER INFORMATION: deletion of signal sequence GK24, alpha-1,5,
 -glucosidase

<400> SEQUENCE: 30

Met	Ser	Trp	Trp	Gln	Arg	Ala	Val	Ile	Tyr	Gln	Val	Tyr	Pro	Arg	Ser
1				5					10					15	
Phe	Gln	Asp	Thr	Asn	Gly	Asp	Gly	Val	Gly	Asp	Leu	Glu	Gly	Ile	Arg
		20						25					30		
Arg	Arg	Leu	Pro	Tyr	Phe	Lys	Ser	Leu	Gly	Val	Asp	Ala	Phe	Trp	Leu
		35					40					45			
Ser	Pro	Phe	Tyr	Lys	Ser	Pro	Met	Lys	Asp	Phe	Gly	Tyr	Asp	Val	Ala
	50					55					60				
Asp	Tyr	Cys	Asp	Val	Asp	Pro	Val	Phe	Gly	Thr	Leu	Gln	Asp	Phe	Asp
65				70					75					80	
Arg	Leu	Leu	Glu	Glu	Ala	His	Ala	Leu	Gly	Leu	Lys	Val	Leu	Val	Asp
			85						90					95	
Leu	Val	Pro	Asn	His	Thr	Ser	Ser	Glu	His	Pro	Trp	Phe	Leu	Glu	Ser
		100						105					110		
Arg	Ala	Ser	Arg	Asn	Ser	Pro	Lys	Arg	Asp	Trp	Tyr	Val	Trp	Lys	Asp
		115					120					125			
Pro	Ala	Pro	Asp	Gly	Gly	Pro	Pro	Asn	Asn	Trp	Gln	Ser	Phe	Phe	Gly
	130					135					140				
Gly	Pro	Ala	Trp	Thr	Leu	Asp	Glu	Ala	Thr	Gly	Gln	Tyr	Tyr	Leu	His
145				150					155					160	
Leu	Phe	Leu	Pro	Glu	Gln	Pro	Asp	Leu	Asn	Trp	Asp	Asn	Pro	Glu	Val
			165					170						175	
Arg	Glu	Ala	Ile	Lys	Glu	Val	Met	Arg	Phe	Trp	Leu	Arg	Arg	Gly	Val
		180						185					190		
Asp	Gly	Phe	Arg	Val	Asp	Val	Leu	Trp	Leu	Leu	Gly	Lys	Asp	Pro	Leu
	195					200					205				
Phe	Arg	Asp	Glu	Pro	Gly	Ser	Pro	Leu	Trp	Arg	Pro	Gly	Leu	Pro	Asp
	210					215					220				
Arg	Ala	Arg	His	Glu	His	Leu	Tyr	Thr	Glu	Asp	Gln	Pro	Glu	Thr	Tyr
225				230					235					240	
Ala	Tyr	Val	Arg	Glu	Met	Arg	Gln	Val	Leu	Asp	Glu	Phe	Ser	Glu	Pro
			245					250						255	
Gly	Arg	Glu	Arg	Val	Met	Val	Gly	Glu	Ile	Tyr	Leu	Pro	Leu	Pro	Arg
		260					265						270		
Leu	Val	Arg	Tyr	Tyr	Ala	Ala	Gly	Cys	His	Leu	Pro	Phe	Asn	Phe	Ser
		275					280					285			
Leu	Val	Thr	Glu	Gly	Leu	Ser	Asp	Trp	Arg	Pro	Glu	Asn	Leu	Ala	Arg
	290					295					300				
Ile	Val	Glu	Thr	Tyr	Glu	Gly	Leu	Leu	Thr	Arg	Trp	Asp	Trp	Pro	Asn
305					310				315					320	
Trp	Val	Leu	Gly	Asn	His	Asp	Gln	Pro	Arg	Leu	Ala	Ser	Arg	Leu	Gly
			325					330						335	
Glu	Pro	Gln	Ala	Arg	Val	Ala	Ala	Met	Leu	Leu	Phe	Thr	Leu	Arg	Gly
		340					345						350		
Thr	Pro	Thr	Trp	Tyr	Tyr	Gly	Asp	Glu	Leu	Ala	Leu	Pro	Asn	Gly	Leu
		355					360					365			
Ile	Pro	Pro	Glu	Lys	Val	Gln	Asp	Pro	Ala	Ala	Leu	Arg	Gln	Arg	Asp
	370					375						380			

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Arg Glu Pro Thr Ala Tyr His Thr Leu Gly Arg Asp Pro Glu Arg Thr
 385 390 395 400
 Pro Met Pro Trp Asp Ala Ser Pro Tyr Gly Gly Phe Ser Thr Val Glu
 405 410 415
 Pro Trp Leu Pro Leu Asn Pro Asp Tyr Lys Thr Arg Asn Val Ala Ala
 420 425 430
 Gln Glu Lys Asp Pro Arg Ser Met Leu His Leu Val Lys Arg Leu Ile
 435 440 445
 Ala Leu Arg Lys Asp Pro Gly Leu Leu Tyr Gly Ala Tyr Arg Thr Tyr
 450 455 460
 Arg Ala Arg Glu Gly Val Tyr Ala Tyr Leu Arg Gly Glu Gly Trp Leu
 465 470 475 480
 Val Ala Leu Asn Leu Thr Glu Lys Glu Lys Ala Leu Glu Leu Pro Arg
 485 490 495
 Gly Gly Arg Val Val Leu Ser Thr His Leu Asp Arg Glu Glu Arg Val
 500 505 510
 Gly Glu Arg Leu Phe Leu Arg Pro Asp Glu Gly Val Ala Val Arg Leu
 515 520 525
 Asp

<210> SEQ ID NO 31
 <211> LENGTH: 575
 <212> TYPE: PRT
 <213> ORGANISM: Thermus thermophilus
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(554)
 <223> OTHER INFORMATION: alpha-1,5-glucosidase

<400> SEQUENCE: 31

Met Val Asp Gly Glu Gly Arg Leu Leu Gly Ile Val Thr Arg Gly Arg
 1 5 10 15
 Leu Leu Ala Ala Leu Ala Gly Arg Tyr Thr Pro Glu Val Pro Gln Ser
 20 25 30
 Gly Val Asp Ser Gly Pro Gln Ser Gly Val Asp Ser Gly Ser Met Ser
 35 40 45
 Trp Trp Gln Arg Ala Val Ile Tyr Gln Val Tyr Pro Arg Ser Phe Gln
 50 55 60
 Asp Thr Asn Gly Asp Gly Val Gly Asp Leu Glu Gly Ile Arg Arg Arg
 65 70 75 80
 Leu Pro Tyr Phe Lys Ser Leu Gly Val Asp Ala Phe Trp Leu Ser Pro
 85 90 95
 Phe Tyr Lys Ser Pro Met Lys Asp Phe Gly Tyr Asp Val Ala Asp Tyr
 100 105 110
 Cys Asp Val Asp Pro Val Phe Gly Thr Leu Gln Asp Phe Asp Arg Leu
 115 120 125
 Leu Glu Glu Ala His Ala Leu Gly Leu Lys Val Leu Val Asp Leu Val
 130 135 140
 Pro Asn His Thr Ser Ser Glu His Pro Trp Phe Leu Glu Ser Arg Ala
 145 150 155 160
 Ser Arg Asn Ser Pro Lys Arg Asp Trp Tyr Val Trp Lys Asp Pro Ala
 165 170 175
 Pro Asp Gly Gly Pro Pro Asn Asn Trp Gln Ser Phe Phe Gly Gly Pro
 180 185 190
 Ala Trp Thr Leu Asp Glu Ala Thr Gly Gln Tyr Tyr Leu His Leu Phe

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195	200	205
Leu Pro Glu Gln Pro Asp 210	Leu Asn Trp Asp Asn 215	Pro Glu Val Arg Glu 220
Ala Ile Lys Glu Val Met 225	Arg Phe Trp Leu Arg 230	Arg Gly Val Asp Gly 235 240
Phe Arg Val Asp Val Leu 245	Trp Leu Leu Gly Lys 250	Asp Pro Leu Phe Arg 255
Asp Glu Pro Gly Ser Pro 260	Leu Trp Arg Pro Gly 265	Leu Pro Asp Arg Ala 270
Arg His Glu His Leu Tyr 275	Thr Glu Asp Gln Pro 280	Glu Thr Tyr Ala Tyr 285
Val Arg Glu Met Arg Gln 290	Val Leu Asp Glu Phe 295	Ser Glu Pro Gly Arg 300
Glu Arg Val Met Val Gly 305	Glu Ile Tyr Leu Pro 310	Leu Pro Arg Leu Val 315 320
Arg Tyr Tyr Ala Ala Gly 325	Cys His Leu Pro Phe 330	Asn Phe Ser Leu Val 335
Thr Glu Gly Leu Ser Asp 340	Trp Arg Pro Glu Asn 345	Leu Ala Arg Ile Val 350
Glu Thr Tyr Glu Gly Leu 355	Leu Thr Arg Trp Asp 360	Trp Pro Asn Trp Val 365
Leu Gly Asn His Asp Gln 370	Pro Arg Leu Ala Ser 375	Arg Leu Gly Glu Pro 380
Gln Ala Arg Val Ala Ala 385	Met Leu Leu Phe Thr 390	Leu Arg Gly Thr Pro 395 400
Thr Trp Tyr Tyr Gly Asp 405	Glu Leu Ala Leu Pro 410	Asn Gly Leu Ile Pro 415
Pro Glu Lys Val Gln Asp 420	Pro Ala Ala Leu Arg 425	Gln Arg Asp Arg Glu 430
Pro Thr Ala Tyr His Thr 435	Leu Gly Arg Asp Pro 440	Glu Arg Thr Pro Met 445
Pro Trp Asp Ala Ser Pro 450	Tyr Gly Gly Phe Ser 455	Thr Val Glu Pro Trp 460
Leu Pro Leu Asn Pro Asp 465	Tyr Lys Thr Arg Asn 470	Val Ala Ala Gln Glu 475 480
Lys Asp Pro Arg Ser Met 485	Leu His Leu Val Lys 490	Arg Leu Ile Ala Leu 495
Arg Lys Asp Pro Gly Leu 500	Leu Tyr Gly Ala Tyr 505	Arg Thr Tyr Arg Ala 510
Arg Glu Gly Val Tyr Ala 515	Tyr Leu Arg Gly Glu 520	Gly Trp Leu Val Ala 525
Leu Asn Leu Thr Glu Lys 530	Glu Lys Ala Leu Glu 535	Leu Pro Arg Gly Gly 540
Arg Val Val Leu Ser Thr 545	His Leu Asp Arg Glu 550	Glu Arg Val Gly Glu 555 560
Arg Leu Phe Leu Arg Pro 565	Asp Glu Gly Val Ala 570	Val Arg Leu Asp 575

<210> SEQ ID NO 32
 <211> LENGTH: 528
 <212> TYPE: PRT
 <213> ORGANISM: Thermus thermophilus
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(507)

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<223> OTHER INFORMATION: alpha-1,5-glucosidase

<400> SEQUENCE: 32

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Met Trp Trp Lys Glu Ala Val Ile Tyr Gln Val Tyr Pro Arg Ser Phe
 1          5          10          15

Gln Asp Thr Asn Gly Asp Gly Val Gly Asp Leu Glu Gly Val Arg Arg
 20          25          30

Arg Leu Pro Tyr Leu Lys Ser Leu Gly Val Asp Ala Leu Trp Leu Ser
 35          40          45

Pro Phe Tyr Lys Ser Pro Met Lys Asp Phe Gly Tyr Asp Val Ala Asp
 50          55          60

Tyr Cys Asp Val Asp Pro Val Phe Gly Thr Leu Gln Asp Phe Asp Arg
 65          70          75          80

Leu Leu Glu Glu Ala His Ala Leu Gly Leu Lys Val Leu Val Asp Leu
 85          90          95

Val Pro Asn His Thr Ser Ser Glu His Pro Trp Phe Leu Glu Ser Arg
 100         105         110

Ala Ser Arg Asn Ser Pro Lys Arg Asp Trp Tyr Ile Trp Lys Asp Pro
 115         120         125

Ala Pro Asp Gly Gly Pro Pro Asn Asn Trp Gln Ser Phe Phe Gly Gly
 130         135         140

Pro Ala Trp Thr Leu Asp Glu Ala Thr Gly Gln Tyr Tyr Leu His Gln
 145         150         155         160

Phe Leu Pro Glu Gln Pro Asp Leu Asn Trp Arg Asn Pro Glu Val Arg
 165         170         175

Glu Ala Ile Tyr Glu Val Met Arg Phe Trp Leu Arg Arg Gly Val Asp
 180         185         190

Gly Phe Arg Val Asp Val Leu Trp Leu Leu Ala Glu Asp Leu Leu Phe
 195         200         205

Arg Asp Glu Pro Gly Asn Pro Asp Trp Arg Pro Gly Met Trp Asp Arg
 210         215         220

Gly Arg His Leu His Ile Phe Thr Glu Asp Gln Pro Glu Thr Tyr Ala
 225         230         235         240

Tyr Val Arg Glu Met Arg Gln Val Leu Asp Glu Phe Ser Glu Pro Gly
 245         250         255

Arg Glu Arg Val Met Val Gly Glu Ile Tyr Leu Pro Tyr Pro Gln Leu
 260         265         270

Val Arg Tyr Tyr Gln Ala Gly Cys His Leu Pro Phe Asn Phe His Leu
 275         280         285

Ile Phe Arg Gly Leu Pro Asp Trp Arg Pro Glu Asn Leu Ala Arg Ile
 290         295         300

Val Glu Glu Tyr Glu Ser Leu Leu Thr Arg Trp Asp Trp Pro Asn Trp
 305         310         315         320

Val Leu Gly Asn His Asp Gln Pro Arg Leu Ala Ser Arg Leu Gly Glu
 325         330         335

Ala Gln Ala Arg Val Ala Ala Met Leu Leu Phe Thr Leu Arg Gly Thr
 340         345         350

Pro Thr Trp Tyr Tyr Gly Asp Glu Ile Gly Met Lys Asn Gly Glu Ile
 355         360         365

Pro Pro Glu Lys Val Gln Asp Pro Ala Ala Leu Arg Gln Lys Asp Arg
 370         375         380

Leu Gly Glu His Asn Leu Pro Pro Gly Arg Asp Pro Glu Arg Thr Pro
 385         390         395         400

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Met Gln Trp Asp Asp Thr Pro Phe Ala Gly Phe Ser Thr Val Glu Pro
405 410 415

Trp Leu Pro Val Asn Pro Asp Tyr Lys Thr Arg Asn Val Ala Ala Gln
420 425 430

Glu Gln Asp Pro Arg Ser Met Leu His Leu Val Arg Arg Leu Ile Ala
435 440 445

Leu Arg Lys Asp Pro Asp Leu Leu Tyr Gly Ala Tyr Arg Thr Tyr Arg
450 455 460

Ala Arg Glu Gly Val Tyr Ala Tyr Leu Arg Gly Glu Gly Trp Leu Val
465 470 475 480

Ala Leu Asn Leu Thr Glu Lys Glu Lys Ala Leu Glu Leu Pro Arg Gly
485 490 495

Gly Arg Val Val Leu Ser Thr His Leu Asp Arg Glu Glu Arg Val Gly
500 505 510

Glu Arg Leu Phe Leu Arg Pro Asp Glu Gly Val Ala Val Arg Leu Asp
515 520 525

<210> SEQ ID NO 33
<211> LENGTH: 529
<212> TYPE: PRT
<213> ORGANISM: Thermus thermophilus
<220> FEATURE:
<221> NAME/KEY: enzyme
<222> LOCATION: (1)..(508)
<223> OTHER INFORMATION: alpha-1,5-glucosidase

<400> SEQUENCE: 33

Met Ser Trp Trp Gln Arg Ala Val Ile Tyr Gln Val Tyr Pro Arg Ser
1 5 10 15

Phe Gln Asp Thr Asn Gly Asp Gly Val Gly Asp Leu Glu Gly Ile Arg
20 25 30

Arg Arg Leu Pro Tyr Leu Lys Ser Leu Gly Val Asp Ala Leu Trp Leu
35 40 45

Ser Pro Phe Tyr Lys Ser Pro Met Lys Asp Phe Gly Tyr Asp Val Ala
50 55 60

Asp Tyr Cys Asp Val Asp Pro Val Phe Gly Thr Leu Gln Asp Phe Asp
65 70 75 80

Arg Leu Leu Glu Glu Ala His Ala Leu Gly Leu Lys Val Leu Val Asp
85 90 95

Leu Val Pro Asn His Thr Ser Ser Glu His Pro Trp Phe Leu Glu Ser
100 105 110

Arg Ala Ser Arg Asn Ser Pro Lys Arg Asp Trp Tyr Ile Trp Lys Asp
115 120 125

Pro Ala Pro Asp Gly Gly Pro Pro Asn Asn Trp Gln Ser Phe Phe Gly
130 135 140

Gly Pro Ala Trp Thr Leu Asp Glu Ala Thr Gly Gln Tyr Tyr Leu His
145 150 155 160

Leu Phe Leu Pro Glu Gln Pro Asp Leu Asn Trp Arg Asn Pro Glu Val
165 170 175

Arg Glu Ala Ile Lys Glu Val Met Arg Phe Trp Leu Arg Arg Gly Val
180 185 190

Asp Gly Phe Arg Val Asp Val Leu Trp Leu Leu Gly Lys Asp Pro Leu
195 200 205

Phe Arg Asp Glu Pro Gly Ser Pro Leu Trp Arg Pro Gly Leu Pro Asp
210 215 220

Arg Ala Arg His Glu His Leu Tyr Thr Glu Asp Gln Pro Glu Thr Tyr

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225	230	235	240
Ala Tyr Val Arg Glu Met Arg Gln Val Leu Asp Glu Phe Ser Glu Pro	245	250	255
Gly Arg Glu Arg Val Met Val Gly Glu Ile Tyr Leu Pro Leu Pro Arg	260	265	270
Leu Val Arg Tyr Tyr Ala Ala Gly Cys His Leu Pro Phe Asn Phe Ser	275	280	285
Leu Val Thr Glu Gly Leu Ser Asp Trp Arg Pro Glu Asn Leu Ala Arg	290	295	300
Ile Val Glu Thr Tyr Glu Gly Leu Leu Ser Arg Trp Asp Trp Pro Asn	305	310	315
Trp Val Leu Gly Asn His Asp Gln Pro Arg Leu Ala Ser Arg Leu Gly	325	330	335
Glu Pro Gln Ala Arg Val Ala Ala Met Leu Leu Phe Thr Leu Arg Gly	340	345	350
Thr Pro Thr Trp Tyr Tyr Gly Asp Glu Leu Ala Leu Pro Asn Gly Leu	355	360	365
Ile Pro Pro Glu Lys Val Gln Asp Pro Ala Ala Leu Arg Gln Arg Asp	370	375	380
Arg Glu Pro Thr Ala Tyr His Thr Leu Gly Arg Asp Pro Glu Arg Thr	385	390	395
Pro Met Pro Trp Asp Ala Ser Pro Tyr Gly Gly Phe Ser Thr Val Glu	405	410	415
Pro Trp Leu Pro Leu Asn Pro Asp Tyr Arg Thr Arg Asn Val Ala Ala	420	425	430
Gln Glu Lys Asp Pro Arg Ser Met Leu His Leu Val Lys Arg Leu Ile	435	440	445
Ala Leu Arg Lys Asp Pro Asp Leu Leu Tyr Gly Ala Tyr Arg Thr Tyr	450	455	460
Arg Ala Arg Glu Gly Val Tyr Ala Tyr Leu Arg Gly Glu Gly Trp Leu	465	470	475
Val Ala Leu Asn Leu Thr Glu Lys Glu Lys Ala Leu Glu Leu Pro Arg	485	490	495
Gly Gly Arg Val Val Leu Ser Thr His Leu Asp Arg Glu Glu Arg Val	500	505	510
Gly Glu Arg Leu Phe Leu Arg Pro Asp Glu Gly Val Ala Val Arg Leu	515	520	525

Asp

<210> SEQ ID NO 34
 <211> LENGTH: 587
 <212> TYPE: PRT
 <213> ORGANISM: Bacillus
 <220> FEATURE:
 <221> NAME/KEY: enzyme
 <222> LOCATION: (1)..(587)
 <223> OTHER INFORMATION: alpha-1,1-glucosidase

<400> SEQUENCE: 34

Met Ser Thr Ala Leu Thr Gln Thr Ser Thr Asn Ser Gln Gln Ser Pro	1	5	10	15
Ile Arg Arg Ala Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro	20	25	30	
Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Ile Gly Asp Leu Arg Gly	35	40	45	

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Ile	Leu	Ser	Lys	Leu	Asp	Tyr	Leu	Lys	Leu	Leu	Gly	Val	Asp	Val	Leu
50						55					60				
Trp	Leu	Asn	Pro	Ile	Tyr	Asp	Ser	Pro	Asn	Asp	Asp	Met	Gly	Tyr	Asp
65					70					75					80
Ile	Arg	Asp	Tyr	Tyr	Lys	Ile	Met	Glu	Glu	Phe	Gly	Thr	Met	Glu	Asp
				85					90					95	
Phe	Glu	Glu	Leu	Leu	Arg	Glu	Val	His	Ala	Arg	Gly	Met	Lys	Leu	Val
			100					105					110		
Met	Asp	Leu	Val	Ala	Asn	His	Thr	Ser	Asp	Glu	His	Pro	Trp	Phe	Ile
		115					120					125			
Glu	Ser	Arg	Ser	Ser	Arg	Asp	Asn	Pro	Tyr	Arg	Asp	Trp	Tyr	Ile	Trp
	130					135					140				
Arg	Asp	Pro	Lys	Asp	Gly	Arg	Glu	Pro	Asn	Asn	Trp	Leu	Ser	Tyr	Phe
145					150					155					160
Ser	Gly	Ser	Ala	Trp	Glu	Tyr	Asp	Glu	Arg	Thr	Gly	Gln	Tyr	Tyr	Leu
				165					170					175	
His	Leu	Phe	Ser	Arg	Arg	Gln	Pro	Asp	Leu	Asn	Trp	Glu	Asn	Pro	Lys
			180					185					190		
Val	Arg	Glu	Ala	Ile	Phe	Glu	Met	Met	Arg	Phe	Trp	Leu	Asp	Lys	Gly
		195					200					205			
Ile	Asp	Gly	Phe	Arg	Met	Asp	Val	Ile	Asn	Ala	Ile	Ala	Lys	Ala	Glu
	210					215					220				
Gly	Leu	Pro	Asp	Ala	Pro	Ala	Arg	Pro	Gly	Glu	Arg	Tyr	Ala	Trp	Gly
225					230					235					240
Gly	Gln	Tyr	Phe	Leu	Asn	Gln	Pro	Lys	Val	His	Glu	Tyr	Leu	Arg	Glu
				245					250					255	
Met	Tyr	Asp	Lys	Val	Leu	Ser	His	Tyr	Asp	Ile	Met	Thr	Val	Gly	Glu
			260					265					270		
Thr	Gly	Gly	Val	Thr	Thr	Lys	Asp	Ala	Leu	Leu	Phe	Ala	Gly	Glu	Asp
		275					280					285			
Arg	Arg	Glu	Leu	Asn	Met	Val	Phe	Gln	Phe	Glu	His	Met	Asp	Ile	Asp
	290					295					300				
Ala	Thr	Asp	Gly	Asp	Lys	Trp	Arg	Pro	Arg	Pro	Trp	Arg	Leu	Thr	Glu
305					310					315					320
Leu	Lys	Thr	Ile	Met	Thr	Arg	Trp	Gln	Asn	Asp	Leu	Tyr	Gly	Lys	Ala
				325					330					335	
Trp	Asn	Ser	Leu	Tyr	Trp	Thr	Asn	His	Asp	Gln	Pro	Arg	Ala	Val	Ser
			340					345					350		
Arg	Phe	Gly	Asn	Asp	Gly	Pro	Tyr	Arg	Val	Glu	Ser	Ala	Lys	Met	Leu
		355					360					365			
Ala	Thr	Val	Leu	His	Met	Met	Gln	Gly	Thr	Pro	Tyr	Ile	Tyr	Gln	Gly
	370					375					380				
Glu	Glu	Ile	Gly	Met	Thr	Asn	Cys	Pro	Phe	Asp	Ser	Ile	Asp	Glu	Tyr
385					390					395					400
Arg	Asp	Val	Glu	Ile	His	Asn	Leu	Trp	Arg	His	Arg	Val	Met	Glu	Gly
				405					410					415	
Gly	Gln	Asp	Pro	Ala	Glu	Val	Leu	Arg	Val	Ile	Gln	Leu	Lys	Gly	Arg
			420					425					430		
Asp	Asn	Ala	Arg	Thr	Pro	Met	Gln	Trp	Asp	Asp	Ser	Pro	Asn	Ala	Gly
		435					440					445			
Phe	Thr	Thr	Gly	Thr	Pro	Trp	Ile	Lys	Val	Asn	Pro	Asn	Tyr	Arg	Glu
	450					455					460				
Ile	Asn	Val	Lys	Gln	Ala	Leu	Ala	Asp	Pro	Asn	Ser	Ile	Phe	His	Tyr

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Tyr Arg Arg Leu Ile Gln Leu Arg Lys Gln His Pro Ile Val Val Tyr			
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Gly Lys Tyr Asp Leu Ile Leu Pro Asp His Glu Glu Ile Trp Ala Tyr			
	500	505	510
Thr Arg Thr Leu Gly Asp Glu Arg Trp Leu Ile Val Ala Asn Phe Phe			
	515	520	525
Gly Gly Thr Pro Glu Phe Glu Leu Pro Pro Glu Val Arg Cys Glu Gly			
	530	535	540
Ala Glu Leu Val Ile Ala Asn Tyr Pro Val Asp Asp Ser Glu Ala Gly			
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gtt caa gaa cca gct act gct caa act tct gga cca gga caa caa act			96
Val Gln Glu Pro Ala Thr Ala Gln Thr Ser Gly Pro Gly Gln Gln Thr			
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cca gct cag gct aag att gct tct gaa caa gag gct gag aaa gtt act			144
Pro Ala Gln Ala Lys Ile Ala Ser Glu Gln Glu Ala Glu Lys Val Thr			
35 40 45			
cca gct gat aag gtg aca gat gat gtt gct gct tct gaa aag cca gct			192
Pro Ala Asp Lys Val Thr Asp Asp Val Ala Ala Ser Glu Lys Pro Ala			
50 55 60			
aaa cca gct gag aat act gag gct act gtt cag act aat gct caa gag			240
Lys Pro Ala Glu Asn Thr Glu Ala Thr Val Gln Thr Asn Ala Gln Glu			
65 70 75 80			
cca gca aag cct gct gat aca aaa gaa gct tcc act gag aag gct gct			288
Pro Ala Lys Pro Ala Asp Thr Lys Glu Ala Ser Thr Glu Lys Ala Ala			
85 90 95			
gtt gct gaa gaa gtt aag gct gct aac gct att act gag atc cca aag			336
Val Ala Glu Glu Val Lys Ala Ala Asn Ala Ile Thr Glu Ile Pro Lys			
100 105 110			
act gaa gtt gct gat cag aat aag caa gct agg cca act act gct caa			384
Thr Glu Val Ala Asp Gln Asn Lys Gln Ala Arg Pro Thr Thr Ala Gln			
115 120 125			
gat caa gag ggt gat aag agg gaa aag act gct gtg gag gat aag att			432
Asp Gln Glu Gly Asp Lys Arg Glu Lys Thr Ala Val Glu Asp Lys Ile			
130 135 140			
gtg gct aac cca aag gtt gca aag aag gat aga ctt cca gaa cca gga			480
Val Ala Asn Pro Lys Val Ala Lys Lys Asp Arg Leu Pro Glu Pro Gly			
145 150 155 160			
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Ser	Lys	Gln	Gly	Ala	Ile	Ala	Glu	Arg	Met	Val	Ala	Asp	Gln	Ala	Gln	
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Pro	Ala	Pro	Val	Asn	Ala	Asp	His	Asp	Asp	Val	Leu	Ser	His	Ile		
			180					185				190				
aag	act	atc	gat	gga	aag	aac	tac	tac	ggt	cag	gat	gat	gga	act	gtg	624
Lys	Thr	Ile	Asp	Gly	Lys	Asn	Tyr	Tyr	Val	Gln	Asp	Asp	Gly	Thr	Val	
		195					200					205				
aag	aag	aac	ttc	gct	ggt	gag	ctt	aac	gga	cgt	atc	ctt	tac	ttt	gat	672
Lys	Lys	Asn	Phe	Ala	Val	Glu	Leu	Asn	Gly	Arg	Ile	Leu	Tyr	Phe	Asp	
	210					215				220						
gct	gag	act	ggt	gct	ctt	ggt	gat	tct	aac	gag	tac	caa	ttc	cag	cag	720
Ala	Glu	Thr	Gly	Ala	Leu	Val	Asp	Ser	Asn	Glu	Tyr	Gln	Phe	Gln	Gln	
	225				230					235					240	
gga	act	tct	tca	ctt	aac	aac	gag	ttc	tcc	cag	aag	aat	gct	ttc	tac	768
Gly	Thr	Ser	Ser	Leu	Asn	Asn	Glu	Phe	Ser	Gln	Lys	Asn	Ala	Phe	Tyr	
			245						250					255		
gga	act	act	gat	aag	gat	atc	gag	act	gtg	gat	gga	tat	ctt	act	gct	816
Gly	Thr	Thr	Asp	Lys	Asp	Ile	Glu	Thr	Val	Asp	Gly	Tyr	Leu	Thr	Ala	
			260					265					270			
gat	tcc	tgg	tat	cgt	cca	aag	ttc	atc	ctc	aag	gat	gga	aag	act	tgg	864
Asp	Ser	Trp	Tyr	Arg	Pro	Lys	Phe	Ile	Leu	Lys	Asp	Gly	Lys	Thr	Trp	
		275					280					285				
act	gct	tcc	act	gaa	act	gat	ctt	agg	cca	ctt	ctt	atg	gct	tgg	tgg	912
Thr	Ala	Ser	Thr	Glu	Thr	Asp	Leu	Arg	Pro	Leu	Leu	Met	Ala	Trp	Trp	
	290					295					300					
cca	gat	aag	agg	act	cag	atc	aac	tac	ctc	aac	tac	atg	aat	cag	caa	960
Pro	Asp	Lys	Arg	Thr	Gln	Ile	Asn	Tyr	Leu	Asn	Tyr	Met	Asn	Gln	Gln	
	305				310					315				320		
gga	ctt	gga	gct	ggt	gct	ttc	gag	aat	aag	gtt	gag	cag	gct	ctt	ttg	1008
Gly	Leu	Gly	Ala	Gly	Ala	Phe	Glu	Asn	Lys	Val	Glu	Gln	Ala	Leu	Leu	
			325						330				335			
act	ggt	gct	tct	caa	caa	ggt	cag	agg	aag	atc	gaa	gag	aag	atc	gga	1056
Thr	Gly	Ala	Ser	Gln	Gln	Val	Gln	Arg	Lys	Ile	Glu	Glu	Lys	Ile	Gly	
			340					345					350			
aaa	gaa	ggt	gat	aca	aag	tgg	ctt	agg	act	ctt	atg	gga	gct	ttc	gtt	1104
Lys	Glu	Gly	Asp	Thr	Lys	Trp	Leu	Arg	Thr	Leu	Met	Gly	Ala	Phe	Val	
		355				360						365				
aag	act	cag	cca	aac	tgg	aac	att	aag	act	gag	tcc	gag	act	act	gga	1152
Lys	Thr	Gln	Pro	Asn	Trp	Asn	Ile	Lys	Thr	Glu	Ser	Glu	Thr	Thr	Gly	
		370				375					380					
act	aag	aag	gat	cat	ctt	cag	gga	ggt	gca	ctt	ctt	tac	act	aac	aac	1200
Thr	Lys	Lys	Asp	His	Leu	Gln	Gly	Gly	Ala	Leu	Leu	Tyr	Thr	Asn	Asn	
	385				390					395					400	
gag	aag	tct	cca	cat	gct	gat	tct	aag	ttc	agg	ctc	ctt	aac	aga	act	1248
Glu	Lys	Ser	Pro	His	Ala	Asp	Ser	Lys	Phe	Arg	Leu	Leu	Asn	Arg	Thr	
			405						410					415		
cca	act	tcc	caa	act	ggt	act	cca	aag	tac	ttc	atc	gat	aag	tcc	aat	1296
Pro	Thr	Ser	Gln	Thr	Gly	Thr	Pro	Lys	Tyr	Phe	Ile	Asp	Lys	Ser	Asn	
			420					425					430			
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Gly	Gly	Tyr	Glu	Phe	Leu	Leu	Ala	Asn	Asp	Phe	Asp	Asn	Ser	Asn	Pro	
		435					440					445				
gcc	gta	caa	gct	gaa	cag	ctt	aac	tgg	ctc	cac	tac	atg	atg	aac	ttc	1392
Ala	Val	Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Tyr	Met	Met	Asn	Phe	
	450					455					460					
gga	tct	atc	gtt	gct	aat	gat	cca	act	gct	aac	ttc	gat	ggt	gtt	aga	1440
Gly	Ser	Ile	Val	Ala	Asn	Asp	Pro	Thr	Ala	Asn	Phe	Asp	Gly	Val	Arg	
	465				470					475					480	

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Val Asp Ala Val Asp Asn Val Asn Ala Asp Leu Leu Gln Ile Ala Ser	
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gat tac ttc aag tcc agg tac aaa gtt gga gaa tct gag gaa gag gct	1536
Asp Tyr Phe Lys Ser Arg Tyr Lys Val Gly Glu Ser Glu Glu Glu Ala	
500 505 510	
att aag cac ctt tct atc ctt gaa gct tgg agt gat aac gat cca gat	1584
Ile Lys His Leu Ser Ile Leu Glu Ala Trp Ser Asp Asn Asp Pro Asp	
515 520 525	
tac aac aag gat aca aag ggt gct cag ttg gct att gat aac aag ctt	1632
Tyr Asn Lys Asp Thr Lys Gly Ala Gln Leu Ala Ile Asp Asn Lys Leu	
530 535 540	
agg ctt tct ctt ctc tac tcc ttc atg agg aac ctt tct att aga tcc	1680
Arg Leu Ser Leu Leu Tyr Ser Phe Met Arg Asn Leu Ser Ile Arg Ser	
545 550 555 560	
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Gly Val Glu Pro Thr Ile Thr Asn Ser Leu Asn Asp Arg Ser Ser Glu	
565 570 575	
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Lys Lys Asn Gly Glu Arg Met Ala Asn Tyr Ile Phe Val Arg Ala His	
580 585 590	
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Asp Asp Glu Val Gln Thr Val Ile Ala Asp Ile Ile Arg Glu Asn Ile	
595 600 605	
aac cca aac act gat gga ctc act ttc act atg gat gag ctt aag cag	1872
Asn Pro Asn Thr Asp Gly Leu Thr Phe Thr Met Asp Glu Leu Lys Gln	
610 615 620	
gct ttc aag atc tac aac gag gat atg agg aag gct gat aag aag tac	1920
Ala Phe Lys Ile Tyr Asn Glu Asp Met Arg Lys Ala Asp Lys Lys Tyr	
625 630 635 640	
act cag ttc aac att cca act gct cac gct ctt atg ctt tcc aac aag	1968
Thr Gln Phe Asn Ile Pro Thr Ala His Ala Leu Met Leu Ser Asn Lys	
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Asp Ser Ile Thr Arg Val Tyr Tyr Gly Asp Leu Tyr Thr Asp Asp Gly	
660 665 670	
cag tac atg gaa aag aag tcc cca tac cac gac gct att gat gct ttg	2064
Gln Tyr Met Glu Lys Lys Ser Pro Tyr His Asp Ala Ile Asp Ala Leu	
675 680 685	
ctc agg gct agg att aag tat gtt gct gga gga cag gat atg aag gtg	2112
Leu Arg Ala Arg Ile Lys Tyr Val Ala Gly Gly Gln Asp Met Lys Val	
690 695 700	
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Thr Tyr Met Gly Val Pro Arg Glu Ala Asp Lys Trp Ser Tyr Asn Gly	
705 710 715 720	
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Ile Leu Thr Ser Val Arg Tyr Gly Thr Gly Ala Asn Glu Ala Thr Asp	
725 730 735	
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Glu Gly Thr Ala Glu Thr Arg Thr Gln Gly Met Ala Val Ile Ala Ser	
740 745 750	
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Asn Asn Pro Asn Leu Lys Leu Asn Glu Trp Asp Lys Leu Gln Val Asn	
755 760 765	
atg gga gct gct cac aag aat cag tac tac cgt cca gtg ctt ctt act	2352
Met Gly Ala Ala His Lys Asn Gln Tyr Tyr Arg Pro Val Leu Leu Thr	
770 775 780	
act aag gat gga atc tcc cgt tat ctc act gat gaa gag gtt cca cag	2400
Thr Lys Asp Gly Ile Ser Arg Tyr Leu Thr Asp Glu Glu Val Pro Gln	
785 790 795 800	

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gct tcc aag aag aag aac gct tct gga caa gtt tac gag tca tct gct Ala Ser Lys Lys Lys Asn Ala Ser Gly Gln Val Tyr Glu Ser Ser Ala 850 855 860	2592
gct ctt gat tct cag ctt atc tac gag gga ttc tcc aat ttt cag gat Ala Leu Asp Ser Gln Leu Ile Tyr Glu Gly Phe Ser Asn Phe Gln Asp 865 870 875 880	2640
ttc gct act agg gat gat cag tac act aac aag gtg atc gct aag aac Phe Ala Thr Arg Asp Asp Gln Tyr Thr Asn Lys Val Ile Ala Lys Asn 885 890 895	2688
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cag tac gtt tct tct cag gat gga act ttc ctc gat tcc atc atc caa Gln Tyr Val Ser Ser Gln Asp Gly Thr Phe Leu Asp Ser Ile Ile Gln 915 920 925	2784
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gat tac ctt act aac agg aac ggt gaa atc gtt ctt cca aag cag Asp Tyr Leu Thr Asn Arg Asn Gly Glu Ile Val Leu Pro Lys Gln 1085 1090 1095	3294
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Phe Ile Gln Asp Glu Asn Gly Asn Trp Tyr Tyr Phe Asp Lys Arg			
1130 1135 1140			
ggt tac ctt gtt act ggt gct cat gag att gat gga aag cac gtg 3474			
Gly Tyr Leu Val Thr Gly Ala His Glu Ile Asp Gly Lys His Val			
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Tyr Phe Leu Lys Asn Gly Ile Gln Leu Arg Asp Ser Ile Arg Glu			
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Lys Ile Val Ser Ala Lys Asp Gly Lys Leu Arg Tyr Phe Asp Lys			
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Asp Ser Gly Asn Ala Val Ile Asn Arg Phe Ala Gln Gly Asp Asn			
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Ile Arg Tyr Phe Asp Ala Asn Ser Gly Glu Met Ala Val Gly Lys			
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Gly Lys Ile Val Thr Leu Ser Asp Lys Ser Ile Arg Tyr Phe Asp	
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Ala Asn Ser Gly Glu Met Ala Thr Asn Lys Phe Val Glu Gly Ser	
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Gln Asn Glu Trp Tyr Tyr Phe Asp Gln Ala Gly Lys Ala Val Thr	
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Gly Leu Gln Gln Val Gly Gln Gln Thr Leu Tyr Phe Thr Gln Asp	
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Gly Lys Gln Val Lys Gly Lys Val Val Asp Val Asn Gly Val Ser	
1490 1495 1500	
cgt tat ttt gac gct aac agc gga gat atg gct cgt tct aag tgg	4554
Arg Tyr Phe Asp Ala Asn Ser Gly Asp Met Ala Arg Ser Lys Trp	
1505 1510 1515	
att cag ctt gag gat ggt tcc tgg atg tat ttc gat agg gat gga	4599
Ile Gln Leu Glu Asp Gly Ser Trp Met Tyr Phe Asp Arg Asp Gly	
1520 1525 1530	
agg gga caa aat ttc ggc agg aac taa	4626
Arg Gly Gln Asn Phe Gly Arg Asn	
1535 1540	

<210> SEQ ID NO 36
 <211> LENGTH: 1541
 <212> TYPE: PRT
 <213> ORGANISM: unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 36

Met Leu Glu Ser Gly Val Val His Ala Asp Asp Val Lys Gln Val Val	
1 5 10 15	
Val Gln Glu Pro Ala Thr Ala Gln Thr Ser Gly Pro Gly Gln Gln Thr	
20 25 30	
Pro Ala Gln Ala Lys Ile Ala Ser Glu Gln Glu Ala Glu Lys Val Thr	
35 40 45	
Pro Ala Asp Lys Val Thr Asp Asp Val Ala Ala Ser Glu Lys Pro Ala	
50 55 60	
Lys Pro Ala Glu Asn Thr Glu Ala Thr Val Gln Thr Asn Ala Gln Glu	
65 70 75 80	
Pro Ala Lys Pro Ala Asp Thr Lys Glu Ala Ser Thr Glu Lys Ala Ala	
85 90 95	
Val Ala Glu Glu Val Lys Ala Ala Asn Ala Ile Thr Glu Ile Pro Lys	
100 105 110	
Thr Glu Val Ala Asp Gln Asn Lys Gln Ala Arg Pro Thr Thr Ala Gln	
115 120 125	
Asp Gln Glu Gly Asp Lys Arg Glu Lys Thr Ala Val Glu Asp Lys Ile	
130 135 140	
Val Ala Asn Pro Lys Val Ala Lys Lys Asp Arg Leu Pro Glu Pro Gly	
145 150 155 160	

Ser 165	Lys	Gln	Gly	Ala	Ile	Ala	Glu	Arg	Met	Val	Ala	Asp	Gln	Ala	Gln
Pro 180	Ala	Pro	Val	Asn	Ala	Asp	His	Asp	Asp	Asp	Val	Leu	Ser	His	Ile
Lys 195	Thr	Ile	Asp	Gly	Lys	Asn	Tyr	Tyr	Val	Gln	Asp	Asp	Gly	Thr	Val
Lys 210	Lys	Asn	Phe	Ala	Val	Glu	Leu	Asn	Gly	Arg	Ile	Leu	Tyr	Phe	Asp
Ala 225	Glu	Thr	Gly	Ala	Leu	Val	Asp	Ser	Asn	Glu	Tyr	Gln	Phe	Gln	Gln
Gly 240	Thr	Ser	Ser	Leu	Asn	Asn	Glu	Phe	Ser	Gln	Lys	Asn	Ala	Phe	Tyr
Gly 255	Thr	Thr	Asp	Lys	Asp	Ile	Glu	Thr	Val	Asp	Gly	Tyr	Leu	Thr	Ala
Asp 270	Ser	Trp	Tyr	Arg	Pro	Lys	Phe	Ile	Leu	Lys	Asp	Gly	Lys	Thr	Trp
Thr 285	Ala	Ser	Thr	Glu	Thr	Asp	Leu	Arg	Pro	Leu	Leu	Met	Ala	Trp	Trp
Pro 300	Asp	Lys	Arg	Thr	Gln	Ile	Asn	Tyr	Leu	Asn	Tyr	Met	Asn	Gln	Gln
Gly 315	Leu	Gly	Ala	Gly	Ala	Phe	Glu	Asn	Lys	Val	Glu	Gln	Ala	Leu	Leu
Thr 330	Gly	Ala	Ser	Gln	Gln	Val	Gln	Arg	Lys	Ile	Glu	Glu	Lys	Ile	Gly
Lys 345	Glu	Gly	Asp	Thr	Lys	Trp	Leu	Arg	Thr	Leu	Met	Gly	Ala	Phe	Val
Lys 360	Thr	Gln	Pro	Asn	Trp	Asn	Ile	Lys	Thr	Glu	Ser	Glu	Thr	Thr	Gly
Thr 375	Lys	Lys	Asp	His	Leu	Gln	Gly	Gly	Ala	Leu	Leu	Tyr	Thr	Asn	Asn
Glu 400	Lys	Ser	Pro	His	Ala	Asp	Ser	Lys	Phe	Arg	Leu	Leu	Asn	Arg	Thr
Pro 415	Thr	Ser	Gln	Thr	Gly	Thr	Pro	Lys	Tyr	Phe	Ile	Asp	Lys	Ser	Asn
Gly 430	Gly	Tyr	Glu	Phe	Leu	Leu	Ala	Asn	Asp	Phe	Asp	Asn	Ser	Asn	Pro
Ala 445	Val	Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Tyr	Met	Met	Asn	Phe
Gly 460	Ser	Ile	Val	Ala	Asn	Asp	Pro	Thr	Ala	Asn	Phe	Asp	Gly	Val	Arg
Val 475	Asp	Ala	Val	Asp	Asn	Val	Asn	Ala	Asp	Leu	Leu	Gln	Ile	Ala	Ser
Asp 490	Tyr	Phe	Lys	Ser	Arg	Tyr	Lys	Val	Gly	Glu	Ser	Glu	Glu	Glu	Ala
Ile 505	Lys	His	Leu	Ser	Ile	Leu	Glu	Ala	Trp	Ser	Asp	Asn	Asp	Pro	Asp
Tyr 520	Asn	Lys	Asp	Thr	Lys	Gly	Ala	Gln	Leu	Ala	Ile	Asp	Asn	Lys	Leu
Arg 535	Leu	Ser	Leu	Leu	Tyr	Ser	Phe	Met	Arg	Asn	Leu	Ser	Ile	Arg	Ser
Gly 550	Val	Glu	Pro	Thr	Ile	Thr	Asn	Ser	Leu	Asn	Asp	Arg	Ser	Ser	Glu
Lys 565	Lys	Asn	Gly	Glu	Arg	Met	Ala	Asn	Tyr	Ile	Phe	Val	Arg	Ala	His

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580						585						590				
Asp	Asp	Glu	Val	Gln	Thr	Val	Ile	Ala	Asp	Ile	Ile	Arg	Glu	Asn	Ile	
		595					600					605				
Asn	Pro	Asn	Thr	Asp	Gly	Leu	Thr	Phe	Thr	Met	Asp	Glu	Leu	Lys	Gln	
	610					615					620					
Ala	Phe	Lys	Ile	Tyr	Asn	Glu	Asp	Met	Arg	Lys	Ala	Asp	Lys	Lys	Tyr	
625					630					635					640	
Thr	Gln	Phe	Asn	Ile	Pro	Thr	Ala	His	Ala	Leu	Met	Leu	Ser	Asn	Lys	
			645					650						655		
Asp	Ser	Ile	Thr	Arg	Val	Tyr	Tyr	Gly	Asp	Leu	Tyr	Thr	Asp	Asp	Gly	
			660					665					670			
Gln	Tyr	Met	Glu	Lys	Lys	Ser	Pro	Tyr	His	Asp	Ala	Ile	Asp	Ala	Leu	
		675					680					685				
Leu	Arg	Ala	Arg	Ile	Lys	Tyr	Val	Ala	Gly	Gly	Gln	Asp	Met	Lys	Val	
	690					695					700					
Thr	Tyr	Met	Gly	Val	Pro	Arg	Glu	Ala	Asp	Lys	Trp	Ser	Tyr	Asn	Gly	
705					710					715					720	
Ile	Leu	Thr	Ser	Val	Arg	Tyr	Gly	Thr	Gly	Ala	Asn	Glu	Ala	Thr	Asp	
			725					730						735		
Glu	Gly	Thr	Ala	Glu	Thr	Arg	Thr	Gln	Gly	Met	Ala	Val	Ile	Ala	Ser	
			740					745					750			
Asn	Asn	Pro	Asn	Leu	Lys	Leu	Asn	Glu	Trp	Asp	Lys	Leu	Gln	Val	Asn	
		755					760					765				
Met	Gly	Ala	Ala	His	Lys	Asn	Gln	Tyr	Tyr	Arg	Pro	Val	Leu	Leu	Thr	
	770				775						780					
Thr	Lys	Asp	Gly	Ile	Ser	Arg	Tyr	Leu	Thr	Asp	Glu	Glu	Val	Pro	Gln	
785					790					795					800	
Ser	Leu	Trp	Lys	Lys	Thr	Asp	Ala	Asn	Gly	Ile	Leu	Thr	Phe	Asp	Met	
			805					810						815		
Asn	Asp	Ile	Ala	Gly	Tyr	Ser	Asn	Val	Gln	Val	Ser	Gly	Tyr	Leu	Ala	
		820					825						830			
Val	Trp	Val	Pro	Val	Gly	Ala	Lys	Ala	Asp	Gln	Asp	Ala	Arg	Thr	Thr	
		835					840					845				
Ala	Ser	Lys	Lys	Lys	Asn	Ala	Ser	Gly	Gln	Val	Tyr	Glu	Ser	Ser	Ala	
	850				855						860					
Ala	Leu	Asp	Ser	Gln	Leu	Ile	Tyr	Glu	Gly	Phe	Ser	Asn	Phe	Gln	Asp	
865					870					875					880	
Phe	Ala	Thr	Arg	Asp	Asp	Gln	Tyr	Thr	Asn	Lys	Val	Ile	Ala	Lys	Asn	
			885					890						895		
Val	Asn	Leu	Phe	Lys	Glu	Trp	Gly	Val	Thr	Ser	Phe	Glu	Leu	Pro	Pro	
		900					905						910			
Gln	Tyr	Val	Ser	Ser	Gln	Asp	Gly	Thr	Phe	Leu	Asp	Ser	Ile	Ile	Gln	
		915				920						925				
Asn	Gly	Tyr	Ala	Phe	Glu	Asp	Arg	Tyr	Asp	Met	Ala	Met	Ser	Lys	Asn	
	930					935					940					
Asn	Lys	Tyr	Gly	Ser	Leu	Lys	Asp	Leu	Leu	Asn	Ala	Leu	Arg	Ala	Leu	
945					950					955					960	
His	Ser	Val	Asn	Ile	Gln	Ala	Ile	Ala	Asp	Trp	Val	Pro	Asp	Gln	Ile	
			965					970						975		
Tyr	Asn	Leu	Pro	Gly	Lys	Glu	Val	Val	Thr	Ala	Thr	Arg	Val	Asn	Asn	
		980						985					990			
Tyr	Gly	Thr	Tyr	Arg	Glu	Gly	Ala	Glu	Ile	Lys	Glu	Lys	Leu	Tyr	Val	
		995					1000						1005			

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Ala Asn	Ser Lys Thr	Asn Glu	Thr Asp Phe Gln Gly	Lys Tyr Gly
1010		1015		1020
Gly Ala	Phe Leu Asp	Glu Leu	Lys Ala Lys Tyr	Pro Glu Ile Phe
1025		1030		1035
Glu Arg	Val Gln Ile	Ser Asn	Gly Gln Lys Met	Thr Thr Asp Glu
1040		1045		1050
Lys Ile	Thr Lys Trp	Ser Ala	Lys Tyr Phe Asn	Gly Thr Asn Ile
1055		1060		1065
Leu Gly	Arg Gly Ala Tyr	Tyr Tyr	Val Leu Lys Asp	Trp Ala Ser Asn
1070		1075		1080
Asp Tyr	Leu Thr Asn Arg	Asn Gly	Glu Ile Val Leu	Pro Lys Gln
1085		1090		1095
Leu Val	Asn Lys Asn Ser	Tyr Thr	Gly Phe Val Ser	Asp Ala Asn
1100		1105		1110
Gly Thr	Lys Phe Tyr Ser	Thr Ser	Gly Tyr Gln Ala	Lys Asn Ser
1115		1120		1125
Phe Ile	Gln Asp Glu Asn	Gly Asn	Trp Tyr Tyr Phe	Asp Lys Arg
1130		1135		1140
Gly Tyr	Leu Val Thr Gly	Ala His	Glu Ile Asp Gly	Lys His Val
1145		1150		1155
Tyr Phe	Leu Lys Asn Gly	Ile Gln	Leu Arg Asp Ser	Ile Arg Glu
1160		1165		1170
Asp Glu	Asn Gly Asn Gln	Tyr Tyr	Tyr Asp Gln Thr	Gly Ala Gln
1175		1180		1185
Val Leu	Asn Arg Tyr Tyr	Thr Thr	Asp Gly Gln Asn	Trp Arg Tyr
1190		1195		1200
Phe Asp	Ala Lys Gly Val	Met Ala	Arg Gly Leu Val	Lys Ile Gly
1205		1210		1215
Asp Gly	Gln Gln Phe Phe	Asp Glu	Asn Gly Tyr Gln	Val Lys Gly
1220		1225		1230
Lys Ile	Val Ser Ala Lys	Asp Gly	Lys Leu Arg Tyr	Phe Asp Lys
1235		1240		1245
Asp Ser	Gly Asn Ala Val	Ile Asn	Arg Phe Ala Gln	Gly Asp Asn
1250		1255		1260
Pro Ser	Asp Trp Tyr Tyr	Phe Gly	Val Glu Phe Ala	Lys Leu Thr
1265		1270		1275
Gly Leu	Gln Lys Ile Gly	Gln Gln	Thr Leu Tyr Phe	Asp Gln Asp
1280		1285		1290
Gly Lys	Gln Val Lys Gly	Lys Ile	Val Thr Leu Ser	Asp Lys Ser
1295		1300		1305
Ile Arg	Tyr Phe Asp Ala	Asn Ser	Gly Glu Met Ala	Val Gly Lys
1310		1315		1320
Phe Ala	Glu Gly Ala Lys	Asn Glu	Trp Tyr Tyr Phe	Asp Lys Thr
1325		1330		1335
Gly Lys	Ala Val Thr Gly	Leu Gln	Lys Ile Gly Lys	Gln Thr Leu
1340		1345		1350
Tyr Phe	Asp Gln Asp Gly	Lys Gln	Val Lys Gly Lys	Val Val Thr
1355		1360		1365
Leu Ala	Asp Lys Ser Ile	Arg Tyr	Phe Asp Ala Asp	Ser Gly Glu
1370		1375		1380
Met Ala	Val Gly Lys Phe	Ala Glu	Gly Ala Lys Asn	Glu Trp Tyr
1385		1390		1395

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Tyr Phe Asp Gln Thr Gly Lys Ala Val Thr Gly Leu Gln Lys Ile
 1400 1405 1410
 Asp Lys Gln Thr Leu Tyr Phe Asp Gln Asp Gly Lys Gln Val Lys
 1415 1420 1425
 Gly Lys Ile Val Thr Leu Ser Asp Lys Ser Ile Arg Tyr Phe Asp
 1430 1435 1440
 Ala Asn Ser Gly Glu Met Ala Thr Asn Lys Phe Val Glu Gly Ser
 1445 1450 1455
 Gln Asn Glu Trp Tyr Tyr Phe Asp Gln Ala Gly Lys Ala Val Thr
 1460 1465 1470
 Gly Leu Gln Gln Val Gly Gln Gln Thr Leu Tyr Phe Thr Gln Asp
 1475 1480 1485
 Gly Lys Gln Val Lys Gly Lys Val Val Asp Val Asn Gly Val Ser
 1490 1495 1500
 Arg Tyr Phe Asp Ala Asn Ser Gly Asp Met Ala Arg Ser Lys Trp
 1505 1510 1515
 Ile Gln Leu Glu Asp Gly Ser Trp Met Tyr Phe Asp Arg Asp Gly
 1520 1525 1530
 Arg Gly Gln Asn Phe Gly Arg Asn
 1535 1540

<210> SEQ ID NO 37
 <211> LENGTH: 4626
 <212> TYPE: DNA
 <213> ORGANISM: unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic gene
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1) .. (4626)
 <223> OTHER INFORMATION: monocot optimized dextranucrase with leucrose
 synthase activity

<400> SEQUENCE: 37

atg ctc gag tct gcc gtg gtg cac gcc gat gac gtg aag cag gtg gtg	48
Met Leu Glu Ser Gly Val Val His Ala Asp Asp Val Lys Gln Val Val	
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gtg caa gag cca gct acc gcc cag acc tct gcc cca ggg cag cag acc	96
Val Gln Glu Pro Ala Thr Ala Gln Thr Ser Gly Pro Gly Gln Gln Thr	
20 25 30	
cct gcc caa gcc aag atc gcg agc gag caa gag gcc gag aag gtg acc	144
Pro Ala Gln Ala Lys Ile Ala Ser Glu Gln Glu Ala Glu Lys Val Thr	
35 40 45	
cca gcc gac aag gtg acc gac gac gtg gcc gcc agc gag aag cca gcc	192
Pro Ala Asp Lys Val Thr Asp Asp Val Ala Ala Ser Glu Lys Pro Ala	
50 55 60	
aag ccg gcc gag aac acc gag gcc acc gtc cag acc aac gcc caa gag	240
Lys Pro Ala Glu Asn Thr Glu Ala Thr Val Gln Thr Asn Ala Gln Glu	
65 70 75 80	
ccg gcc aag ccc gcc gac acc aag gaa gcc agc acc gag aag gcc gcc	288
Pro Ala Lys Pro Ala Asp Thr Lys Glu Ala Ser Thr Glu Lys Ala Ala	
85 90 95	
gtg gcc gag gaa gtg aag gcc gcc aac gcc atc acc gag atc ccc aag	336
Val Ala Glu Glu Val Lys Ala Ala Asn Ala Ile Thr Glu Ile Pro Lys	
100 105 110	
acc gag gtg gcc gac cag aac aag cag gcc agg ccg acc acc gct cag	384
Thr Glu Val Ala Asp Gln Asn Lys Gln Ala Arg Pro Thr Thr Ala Gln	
115 120 125	
gac caa gag ggc gac aag cgc gaa aag acc gcc gtg gag gac aag atc	432
Asp Gln Glu Gly Asp Lys Arg Glu Lys Thr Ala Val Glu Asp Lys Ile	

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130	135	140	
gtg gcc aac ccg aag gtg gcc aag aag gac agg ctg ccc gag cca ggc Val Ala Asn Pro Lys Val Ala Lys Lys Asp Arg Leu Pro Glu Pro Gly 145 150 155 160			480
agc aag cag ggc gcg atc gcc gag agg atg gtc gcc gac cag gcc cag Ser Lys Gln Gly Ala Ile Ala Glu Arg Met Val Ala Asp Gln Ala Gln 165 170 175			528
cca gcc cca gtg aac gcc gac cac gac gat gat gtg ctg tcc cac atc Pro Ala Pro Val Asn Ala Asp His Asp Asp Val Leu Ser His Ile 180 185 190			576
aag acc atc gac ggc aag aac tac tac gtc cag gac gac ggc acc gtg Lys Thr Ile Asp Gly Lys Asn Tyr Tyr Val Gln Asp Asp Gly Thr Val 195 200 205			624
aag aag aac ttc gcc gtc gag ctg aac ggc cgc atc ctg tac ttc gac Lys Lys Asn Phe Ala Val Glu Leu Asn Gly Arg Ile Leu Tyr Phe Asp 210 215 220			672
gcc gag act ggc gcc ctg gtg gac agc aac gag tac cag ttc cag cag Ala Glu Thr Gly Ala Leu Val Asp Ser Asn Glu Tyr Gln Phe Gln Gln 225 230 235 240			720
ggc acc agc agc ctg aac aac gag ttc agc cag aag aac gcc ttc tac Gly Thr Ser Ser Leu Asn Asn Glu Phe Ser Gln Lys Asn Ala Phe Tyr 245 250 255			768
ggc acc acc gac aag gac atc gag act gtg gac ggc tac ctg acc gcc Gly Thr Thr Asp Lys Asp Ile Glu Thr Val Asp Gly Tyr Leu Thr Ala 260 265 270			816
gac agc tgg tat cgc ccg aag ttc atc ctg aag gac ggc aag acc tgg Asp Ser Trp Tyr Arg Pro Lys Phe Ile Leu Lys Asp Gly Lys Thr Trp 275 280 285			864
acc gcc tcc acc gag act gac ctg cgc ccg ctg ctg atg gcc tgg tgg Thr Ala Ser Thr Glu Thr Asp Leu Arg Pro Leu Leu Met Ala Trp Trp 290 295 300			912
ccg gac aag cgc acc cag atc aac tac ctg aac tac atg aac cag caa Pro Asp Lys Arg Thr Gln Ile Asn Tyr Leu Asn Tyr Met Asn Gln Gln 305 310 315 320			960
ggc ctg ggc gct ggc gct ttc gag aac aag gtg gag cag gcc ctc ctg Gly Leu Gly Ala Gly Ala Phe Glu Asn Lys Val Glu Gln Ala Leu Leu 325 330 335			1008
acc ggc gct agc cag cag gtc cag cgc aag atc gag gaa aag atc ggc Thr Gly Ala Ser Gln Gln Val Gln Arg Lys Ile Glu Glu Lys Ile Gly 340 345 350			1056
aag gaa ggc gac acc aag tgg ctg cgc acc ctg atg ggc gcc ttc gtc Lys Glu Gly Asp Thr Lys Trp Leu Arg Thr Leu Met Gly Ala Phe Val 355 360 365			1104
aag acc cag ccg aac tgg aac atc aag acc gag agc gag act acc ggc Lys Thr Gln Pro Asn Trp Asn Ile Lys Thr Glu Ser Glu Thr Thr Gly 370 375 380			1152
acc aag aag gac cac ctc cag ggc ggt gcc ctg ctg tac acc aac aac Thr Lys Lys Asp His Leu Gln Gly Gly Ala Leu Leu Tyr Thr Asn Asn 385 390 395 400			1200
gag aag tct ccg cac gcc gac agc aag ttc cgc ctg ctg aac cgc acc Glu Lys Ser Pro His Ala Asp Ser Lys Phe Arg Leu Leu Asn Arg Thr 405 410 415			1248
cca acc agc cag acc ggc acc ccg aag tac ttc atc gac aag agc aac Pro Thr Ser Gln Thr Gly Thr Pro Lys Tyr Phe Ile Asp Lys Ser Asn 420 425 430			1296
ggc ggc tac gag ttc ctg ctg gcc aac gac ttc gac aac agc aac ccg Gly Gly Tyr Glu Phe Leu Leu Ala Asn Asp Phe Asp Asn Ser Asn Pro 435 440 445			1344
gcc gtc cag gcc gag cag ctg aac tgg ctg cac tac atg atg aac ttc			1392

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Ala	Val	Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Tyr	Met	Met	Asn	Phe	
450						455					460					
ggc	agc	atc	gtc	gcc	aac	gac	ccg	acc	gcc	aac	ttc	gac	ggt	gtc	cgc	1440
Gly	Ser	Ile	Val	Ala	Asn	Asp	Pro	Thr	Ala	Asn	Phe	Asp	Gly	Val	Arg	
465					470					475					480	
gtg	gac	gcc	gtg	gac	aac	gtc	aac	gcc	gac	ctg	ctc	cag	atc	gcc	tcc	1488
Val	Asp	Ala	Val	Asp	Asn	Val	Asn	Ala	Asp	Leu	Leu	Gln	Ile	Ala	Ser	
				485					490					495		
gac	tac	ttc	aag	agc	cgc	tac	aaa	gtg	ggc	gag	agc	gag	gaa	gag	gcc	1536
Asp	Tyr	Phe	Lys	Ser	Arg	Tyr	Lys	Val	Gly	Glu	Ser	Glu	Glu	Glu	Ala	
			500					505					510			
atc	aag	cac	ctg	agc	atc	ctc	gag	gcg	tgg	agc	gac	aac	gac	ccg	gac	1584
Ile	Lys	His	Leu	Ser	Ile	Leu	Glu	Ala	Trp	Ser	Asp	Asn	Asp	Pro	Asp	
		515					520					525				
tac	aac	aag	gac	acc	aag	ggc	gcc	cag	ctg	gcc	atc	gac	aac	aag	ctg	1632
Tyr	Asn	Lys	Asp	Thr	Lys	Gly	Ala	Gln	Leu	Ala	Ile	Asp	Asn	Lys	Leu	
	530					535					540					
cgc	ctg	agc	ctg	ctg	tac	tcc	ttc	atg	cgc	aac	ctg	tcc	atc	cgc	agc	1680
Arg	Leu	Ser	Leu	Leu	Tyr	Ser	Phe	Met	Arg	Asn	Leu	Ser	Ile	Arg	Ser	
	545				550					555					560	
ggc	gtc	gag	ccg	acc	atc	acc	aac	agc	ctc	aac	gac	cgc	agc	agc	gag	1728
Gly	Val	Glu	Pro	Thr	Ile	Thr	Asn	Ser	Leu	Asn	Asp	Arg	Ser	Ser	Glu	
				565					570					575		
aag	aaa	aac	ggc	gag	cgc	atg	gcc	aac	tac	atc	ttt	gtc	cgc	gct	cac	1776
Lys	Lys	Asn	Gly	Glu	Arg	Met	Ala	Asn	Tyr	Ile	Phe	Val	Arg	Ala	His	
		580						585					590			
gac	gac	gag	gtc	cag	acc	gtg	atc	gcc	gac	atc	atc	cgc	gag	aac	atc	1824
Asp	Asp	Glu	Val	Gln	Thr	Val	Ile	Ala	Asp	Ile	Ile	Arg	Glu	Asn	Ile	
		595					600					605				
aac	ccg	aac	acc	gac	ggc	ctg	acc	ttc	acc	atg	gac	gag	ctg	aag	cag	1872
Asn	Pro	Asn	Thr	Asp	Gly	Leu	Thr	Phe	Thr	Met	Asp	Glu	Leu	Lys	Gln	
	610					615					620					
gcc	ttc	aag	atc	tac	aac	gag	gac	atg	cgc	aag	gcc	gac	aag	aag	tac	1920
Ala	Phe	Lys	Ile	Tyr	Asn	Glu	Asp	Met	Arg	Lys	Ala	Asp	Lys	Lys	Tyr	
	625				630					635					640	
acc	cag	ttc	aac	atc	ccg	acc	gcc	cac	gcc	ctg	atg	ctg	tcc	aac	aag	1968
Thr	Gln	Phe	Asn	Ile	Pro	Thr	Ala	His	Ala	Leu	Met	Leu	Ser	Asn	Lys	
			645						650					655		
gac	agc	atc	acc	cgc	gtg	tac	tac	ggc	gac	ctg	tac	acc	gac	gac	ggc	2016
Asp	Ser	Ile	Thr	Arg	Val	Tyr	Tyr	Gly	Asp	Leu	Tyr	Thr	Asp	Asp	Gly	
		660						665					670			
cag	tac	atg	gaa	aag	aag	tcc	ccg	tac	cac	gac	gcc	atc	gac	gcc	ctg	2064
Gln	Tyr	Met	Glu	Lys	Lys	Ser	Pro	Tyr	His	Asp	Ala	Ile	Asp	Ala	Leu	
		675					680					685				
ctg	agg	gcc	cgc	atc	aag	tac	gtg	gct	ggc	ggc	cag	gac	atg	aag	gtg	2112
Leu	Arg	Ala	Arg	Ile	Lys	Tyr	Val	Ala	Gly	Gly	Gln	Asp	Met	Lys	Val	
	690					695					700					
acc	tac	atg	ggc	gtg	ccc	cgc	gag	gcc	gac	aag	tgg	agc	tac	aac	ggc	2160
Thr	Tyr	Met	Gly	Val	Pro	Arg	Glu	Ala	Asp	Lys	Trp	Ser	Tyr	Asn	Gly	
	705				710					715					720	
atc	ctg	acc	tct	gtg	cgc	tac	ggc	acc	ggc	gcc	aac	gag	gct	acc	gac	2208
Ile	Leu	Thr	Ser	Val	Arg	Tyr	Gly	Thr	Gly	Ala	Asn	Glu	Ala	Thr	Asp	
			725						730					735		
gag	ggc	acc	gcc	gag	act	agg	acc	cag	ggc	atg	gcc	gtg	atc	gcc	agc	2256
Glu	Gly	Thr	Ala	Glu	Thr	Arg	Thr	Gln	Gly	Met	Ala	Val	Ile	Ala	Ser	
		740						745					750			
aac	aac	ccg	aac	ctg	aag	ctg	aac	gag	tgg	gac	aag	ctc	cag	gtg	aac	2304
Asn	Asn	Pro	Asn	Leu	Lys	Leu	Asn	Glu	Trp	Asp	Lys	Leu	Gln	Val	Asn	
		755					760						765			

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atg ggc gct gcc cac aag aac cag tac tac cgc ccg gtg ctg ctg acc	2352
Met Gly Ala Ala His Lys Asn Gln Tyr Tyr Arg Pro Val Leu Leu Thr	
770 775 780	
acc aag gac ggc atc tcg cgc tac ctc acc gac gag gaa gtg ccg cag	2400
Thr Lys Asp Gly Ile Ser Arg Tyr Leu Thr Asp Glu Glu Val Pro Gln	
785 790 795 800	
agc ctc tgg aag aaa acc gac gcg aac ggc atc ctc act ttc gac atg	2448
Ser Leu Trp Lys Thr Asp Ala Asn Gly Ile Leu Thr Phe Asp Met	
805 810 815	
aac gat atc gcc ggc tac tcc aac gtc cag gtg tcc ggc tac ctg gct	2496
Asn Asp Ile Ala Gly Tyr Ser Asn Val Gln Val Ser Gly Tyr Leu Ala	
820 825 830	
gtg tgg gtg cca gtg ggc gcc aag gcc gac cag gac gcc agg acc acc	2544
Val Trp Val Pro Val Gly Ala Lys Ala Asp Gln Asp Ala Arg Thr Thr	
835 840 845	
gcc tcc aag aag aag aac gcc agc ggc cag gtg tac gag agc agc gcc	2592
Ala Ser Lys Lys Lys Asn Ala Ser Gly Gln Val Tyr Glu Ser Ser Ala	
850 855 860	
gct ctg gac agc cag ctg atc tac gag ggc ttc agc aac ttc cag gac	2640
Ala Leu Asp Ser Gln Leu Ile Tyr Glu Gly Phe Ser Asn Phe Gln Asp	
865 870 875 880	
ttc gcg acc cgc gac gac cag tac acg aac aag gtg atc gcc aag aac	2688
Phe Ala Thr Arg Asp Asp Gln Tyr Thr Asn Lys Val Ile Ala Lys Asn	
885 890 895	
gtg aac ctg ttc aag gaa tgg ggc gtg acc agc ttc gag ctg ccg ccg	2736
Val Asn Leu Phe Lys Glu Trp Gly Val Thr Ser Phe Glu Leu Pro Pro	
900 905 910	
cag tac gtg tct agc cag gac ggc acc ttc ctg gac agc atc atc cag	2784
Gln Tyr Val Ser Ser Gln Asp Gly Thr Phe Leu Asp Ser Ile Ile Gln	
915 920 925	
aac ggc tac gcc ttc gag gac cgc tac gac atg gcc atg agc aag aac	2832
Asn Gly Tyr Ala Phe Glu Asp Arg Tyr Asp Met Ala Met Ser Lys Asn	
930 935 940	
aac aag tac ggc agc ctg aag gac ctg ctg aac gcc ctg cgc gcc ctg	2880
Asn Lys Tyr Gly Ser Leu Lys Asp Leu Leu Asn Ala Leu Arg Ala Leu	
945 950 955 960	
cac agc gtg aac atc cag gcg atc gct gac tgg gtg ccg gac cag atc	2928
His Ser Val Asn Ile Gln Ala Ile Ala Asp Trp Val Pro Asp Gln Ile	
965 970 975	
tac aac ctg ccg ggc aag gaa gtg gtg acc gcc acc cgc gtg aac aac	2976
Tyr Asn Leu Pro Gly Lys Glu Val Val Thr Ala Thr Arg Val Asn Asn	
980 985 990	
tac ggc acc tac cgc gag ggc gcc gag atc aag gaa aag ctg tac gtc	3024
Tyr Gly Thr Tyr Arg Glu Gly Ala Glu Ile Lys Glu Lys Leu Tyr Val	
995 1000 1005	
gcc aac agc aag acc aac gag act gac ttc cag ggc aag tac ggc	3069
Ala Asn Ser Lys Thr Asn Glu Thr Asp Phe Gln Gly Lys Tyr Gly	
1010 1015 1020	
ggg gcc ttc ctg gat gag ctg aag gcc aag tac ccc gag atc ttc	3114
Gly Ala Phe Leu Asp Glu Leu Lys Ala Lys Tyr Pro Glu Ile Phe	
1025 1030 1035	
gag cgc gtc cag atc agc aac ggc cag aag atg acc acc gac gag	3159
Glu Arg Val Gln Ile Ser Asn Gly Gln Lys Met Thr Thr Asp Glu	
1040 1045 1050	
aag atc acc aag tgg agc gcc aag tac ttc aac ggc acc aac atc	3204
Lys Ile Thr Lys Trp Ser Ala Lys Tyr Phe Asn Gly Thr Asn Ile	
1055 1060 1065	
ctg ggc agg ggc gcc tac tac gtg ctg aag gac tgg gcc agc aac	3249
Leu Gly Arg Gly Ala Tyr Tyr Val Leu Lys Asp Trp Ala Ser Asn	
1070 1075 1080	

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gac tac	ctg acc aac cgc aac	ggc gag atc gtg ctg	ccg aag cag	3294
Asp Tyr	Leu Thr Asn Arg Asn	Gly Glu Ile Val Leu	Pro Lys Gln	
1085	1090	1095		
ctg gtg	aac aag aac agc tac	acc ggc ttc gtg tcc	gac gcc aac	3339
Leu Val	Asn Lys Asn Ser Tyr	Thr Gly Phe Val Ser	Asp Ala Asn	
1100	1105	1110		
ggc acg	aag ttc tac tcc acc	tcc ggg tat caa gcc	aag aac agc	3384
Gly Thr	Lys Phe Tyr Ser Thr	Ser Gly Tyr Gln Ala	Lys Asn Ser	
1115	1120	1125		
ttc atc	caa gac gag aat ggc	aac tgg tac tac ttc	gac aag cgc	3429
Phe Ile	Gln Asp Glu Asn Gly	Asn Trp Tyr Tyr Phe	Asp Lys Arg	
1130	1135	1140		
ggc tac	ctg gtg acc ggc gct	cac gag atc gac ggg	aag cac gtg	3474
Gly Tyr	Leu Val Thr Gly Ala	His Glu Ile Asp Gly	Lys His Val	
1145	1150	1155		
tac ttt	ctg aag aac ggc atc	cag ctg cgc gac tcc	atc cgc gag	3519
Tyr Phe	Leu Lys Asn Gly Ile	Gln Leu Arg Asp Ser	Ile Arg Glu	
1160	1165	1170		
gat gag	aac ggg aat cag tac	tac tac gac cag aca	ggc gcc cag	3564
Asp Glu	Asn Gly Asn Gln Tyr	Tyr Tyr Asp Gln Thr	Gly Ala Gln	
1175	1180	1185		
gtg ctg	aac cgc tac tac acc	acc gac ggc cag aac	tgg cgc tac	3609
Val Leu	Asn Arg Tyr Tyr Thr	Thr Asp Gly Gln Asn	Trp Arg Tyr	
1190	1195	1200		
ttc gac	gcg aag ggc gtg atg	gcc agg ggc ctg gtg	aag atc ggc	3654
Phe Asp	Ala Lys Gly Val Met	Ala Arg Gly Leu Val	Lys Ile Gly	
1205	1210	1215		
gac ggc	cag cag ttc ttc gat	gag aac ggc tac caa	gtg aag ggc	3699
Asp Gly	Gln Gln Phe Phe Asp	Glu Asn Gly Tyr Gln	Val Lys Gly	
1220	1225	1230		
aag atc	gtg tcc gcc aag gac	ggg aag ctc cgc tac	ttc gat aag	3744
Lys Ile	Val Ser Ala Lys Asp	Gly Lys Leu Arg Tyr	Phe Asp Lys	
1235	1240	1245		
gac agc	ggc aac gcc gtg atc	aac cgc ttc gct cag	ggc gac aac	3789
Asp Ser	Gly Asn Ala Val Ile	Asn Arg Phe Ala Gln	Gly Asp Asn	
1250	1255	1260		
ccg agc	gat tgg tac tac ttt	ggc gtg gag ttc gcc	aag ctg acc	3834
Pro Ser	Asp Trp Tyr Tyr Phe	Gly Val Glu Phe Ala	Lys Leu Thr	
1265	1270	1275		
ggc ctc	cag aag att ggc cag	cag acg ctc tac ttc	gac cag gac	3879
Gly Leu	Gln Lys Ile Gly Gln	Gln Thr Leu Tyr Phe	Asp Gln Asp	
1280	1285	1290		
ggc aag	cag gtc aag ggg aag	att gtc acc ctg agc	gac aag tcg	3924
Gly Lys	Gln Val Lys Gly Lys	Ile Val Thr Leu Ser	Asp Lys Ser	
1295	1300	1305		
atc cgc	tac ttt gat gcc aac	agc ggc gag atg gct	gtg ggc aag	3969
Ile Arg	Tyr Phe Asp Ala Asn	Ser Gly Glu Met Ala	Val Gly Lys	
1310	1315	1320		
ttc gcc	gag ggc gcc aag aac	gag tgg tac tac ttt	gat aag acc	4014
Phe Ala	Glu Gly Ala Lys Asn	Glu Trp Tyr Tyr Phe	Asp Lys Thr	
1325	1330	1335		
ggc aag	gcc gtc acc ggg ctg	caa aag atc ggg aag	cag acc ctg	4059
Gly Lys	Ala Val Thr Gly Leu	Gln Lys Ile Gly Lys	Gln Thr Leu	
1340	1345	1350		
tac ttt	gat cag gat ggg aag	caa gtt aag ggc aag	gtg gtg acc	4104
Tyr Phe	Asp Gln Asp Gly Lys	Gln Val Lys Gly Lys	Val Val Thr	
1355	1360	1365		
ctg gcc	gac aag agc atc aga	tac ttc gac gct gac	tcg ggt gaa	4149
Leu Ala	Asp Lys Ser Ile Arg	Tyr Phe Asp Ala Asp	Ser Gly Glu	

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1370	1375	1380	
atg gct gtc ggt aag ttt gcg gaa ggg gcg aag aat gaa tgg tat			4194
Met Ala Val Gly Lys Phe Ala Glu Gly Ala Lys Asn Glu Trp Tyr			
1385	1390	1395	
tat ttc gat caa act ggg aag gcg gtg acg ggg ctt cag aag atc			4239
Tyr Phe Asp Gln Thr Gly Lys Ala Val Thr Gly Leu Gln Lys Ile			
1400	1405	1410	
gac aag caa acg ttg tac ttc gac caa gac ggg aag caa gtg aag			4284
Asp Lys Gln Thr Leu Tyr Phe Asp Gln Asp Gly Lys Gln Val Lys			
1415	1420	1425	
ggc aag att gtg acc ctc tcc gac aag tcc att cgg tac ttc gat			4329
Gly Lys Ile Val Thr Leu Ser Asp Lys Ser Ile Arg Tyr Phe Asp			
1430	1435	1440	
gcc aac tcc ggg gag atg gcc acc aac aag ttc gtg gag ggc tcg			4374
Ala Asn Ser Gly Glu Met Ala Thr Asn Lys Phe Val Glu Gly Ser			
1445	1450	1455	
cag aat gag tgg tac tac ttc gac cag gct ggc aag gct gtg acc			4419
Gln Asn Glu Trp Tyr Tyr Phe Asp Gln Ala Gly Lys Ala Val Thr			
1460	1465	1470	
ggc ctt cag caa gtg ggg cag caa act ctg tac ttc acc caa gat			4464
Gly Leu Gln Gln Val Gly Gln Gln Thr Leu Tyr Phe Thr Gln Asp			
1475	1480	1485	
ggc aag caa gtc aag ggc aag gtc gtg gac gtg aac ggc gtg tcc			4509
Gly Lys Gln Val Lys Gly Lys Val Val Asp Val Asn Gly Val Ser			
1490	1495	1500	
cgg tac ttt gac gcg aac tct ggc gac atg gcc cgc agc aag tgg			4554
Arg Tyr Phe Asp Ala Asn Ser Gly Asp Met Ala Arg Ser Lys Trp			
1505	1510	1515	
att cag ctc gag gac ggc agc tgg atg tac ttc gat cgc gac ggc			4599
Ile Gln Leu Glu Asp Gly Ser Trp Met Tyr Phe Asp Arg Asp Gly			
1520	1525	1530	
agg ggc cag aac ttc ggc cgc aac tga			4626
Arg Gly Gln Asn Phe Gly Arg Asn			
1535	1540		

<210> SEQ ID NO 38

<211> LENGTH: 1541

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 38

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Val Gln Glu Pro Ala Thr Ala Gln Thr Ser Gly Pro Gly Gln Gln Thr
20 25 30

Pro Ala Gln Ala Lys Ile Ala Ser Glu Gln Glu Ala Glu Lys Val Thr
35 40 45

Pro Ala Asp Lys Val Thr Asp Asp Val Ala Ala Ser Glu Lys Pro Ala
50 55 60

Lys Pro Ala Glu Asn Thr Glu Ala Thr Val Gln Thr Asn Ala Gln Glu
65 70 75 80

Pro Ala Lys Pro Ala Asp Thr Lys Glu Ala Ser Thr Glu Lys Ala Ala
85 90 95

Val Ala Glu Glu Val Lys Ala Ala Asn Ala Ile Thr Glu Ile Pro Lys
100 105 110

Thr Glu Val Ala Asp Gln Asn Lys Gln Ala Arg Pro Thr Thr Ala Gln
115 120 125

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Asp	Gln	Glu	Gly	Asp	Lys	Arg	Glu	Lys	Thr	Ala	Val	Glu	Asp	Lys	Ile
130						135					140				
Val	Ala	Asn	Pro	Lys	Val	Ala	Lys	Lys	Asp	Arg	Leu	Pro	Glu	Pro	Gly
145				150					155						160
Ser	Lys	Gln	Gly	Ala	Ile	Ala	Glu	Arg	Met	Val	Ala	Asp	Gln	Ala	Gln
				165				170						175	
Pro	Ala	Pro	Val	Asn	Ala	Asp	His	Asp	Asp	Asp	Val	Leu	Ser	His	Ile
			180				185						190		
Lys	Thr	Ile	Asp	Gly	Lys	Asn	Tyr	Tyr	Val	Gln	Asp	Asp	Gly	Thr	Val
		195				200					205				
Lys	Lys	Asn	Phe	Ala	Val	Glu	Leu	Asn	Gly	Arg	Ile	Leu	Tyr	Phe	Asp
210					215					220					
Ala	Glu	Thr	Gly	Ala	Leu	Val	Asp	Ser	Asn	Glu	Tyr	Gln	Phe	Gln	Gln
225					230					235					240
Gly	Thr	Ser	Ser	Leu	Asn	Asn	Glu	Phe	Ser	Gln	Lys	Asn	Ala	Phe	Tyr
				245				250						255	
Gly	Thr	Thr	Asp	Lys	Asp	Ile	Glu	Thr	Val	Asp	Gly	Tyr	Leu	Thr	Ala
			260					265					270		
Asp	Ser	Trp	Tyr	Arg	Pro	Lys	Phe	Ile	Leu	Lys	Asp	Gly	Lys	Thr	Trp
		275				280					285				
Thr	Ala	Ser	Thr	Glu	Thr	Asp	Leu	Arg	Pro	Leu	Leu	Met	Ala	Trp	Trp
		290				295					300				
Pro	Asp	Lys	Arg	Thr	Gln	Ile	Asn	Tyr	Leu	Asn	Tyr	Met	Asn	Gln	Gln
305					310					315					320
Gly	Leu	Gly	Ala	Gly	Ala	Phe	Glu	Asn	Lys	Val	Glu	Gln	Ala	Leu	Leu
				325					330					335	
Thr	Gly	Ala	Ser	Gln	Gln	Val	Gln	Arg	Lys	Ile	Glu	Glu	Lys	Ile	Gly
			340					345					350		
Lys	Glu	Gly	Asp	Thr	Lys	Trp	Leu	Arg	Thr	Leu	Met	Gly	Ala	Phe	Val
		355				360					365				
Lys	Thr	Gln	Pro	Asn	Trp	Asn	Ile	Lys	Thr	Glu	Ser	Glu	Thr	Thr	Gly
		370				375					380				
Thr	Lys	Lys	Asp	His	Leu	Gln	Gly	Gly	Ala	Leu	Leu	Tyr	Thr	Asn	Asn
385					390					395					400
Glu	Lys	Ser	Pro	His	Ala	Asp	Ser	Lys	Phe	Arg	Leu	Leu	Asn	Arg	Thr
				405					410					415	
Pro	Thr	Ser	Gln	Thr	Gly	Thr	Pro	Lys	Tyr	Phe	Ile	Asp	Lys	Ser	Asn
			420					425					430		
Gly	Gly	Tyr	Glu	Phe	Leu	Leu	Ala	Asn	Asp	Phe	Asp	Asn	Ser	Asn	Pro
		435					440					445			
Ala	Val	Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	His	Tyr	Met	Met	Asn	Phe
		450				455					460				
Gly	Ser	Ile	Val	Ala	Asn	Asp	Pro	Thr	Ala	Asn	Phe	Asp	Gly	Val	Arg
465					470					475					480
Val	Asp	Ala	Val	Asp	Asn	Val	Asn	Ala	Asp	Leu	Leu	Gln	Ile	Ala	Ser
				485					490					495	
Asp	Tyr	Phe	Lys	Ser	Arg	Tyr	Lys	Val	Gly	Glu	Ser	Glu	Glu	Glu	Ala
			500					505					510		
Ile	Lys	His	Leu	Ser	Ile	Leu	Glu	Ala	Trp	Ser	Asp	Asn	Asp	Pro	Asp
			515					520				525			
Tyr	Asn	Lys	Asp	Thr	Lys	Gly	Ala	Gln	Leu	Ala	Ile	Asp	Asn	Lys	Leu
			530			535					540				

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Arg 545	Leu	Ser	Leu	Leu	Tyr 550	Ser	Phe	Met	Arg	Asn 555	Leu	Ser	Ile	Arg	Ser 560
Gly	Val	Glu	Pro	Thr 565	Ile	Thr	Asn	Ser	Leu 570	Asn	Asp	Arg	Ser	Ser	Glu 575
Lys	Lys	Asn 580	Gly	Glu	Arg	Met	Ala	Asn 585	Tyr	Ile	Phe	Val	Arg 590	Ala	His
Asp	Asp	Glu 595	Val	Gln	Thr	Val	Ile 600	Ala	Asp	Ile	Ile	Arg 605	Glu	Asn	Ile
Asn 610	Pro	Asn	Thr	Asp	Gly	Leu 615	Thr	Phe	Thr	Met	Asp 620	Glu	Leu	Lys	Gln
Ala 625	Phe	Lys	Ile	Tyr	Asn 630	Glu	Asp	Met	Arg	Lys 635	Ala	Asp	Lys	Lys	Tyr 640
Thr	Gln	Phe	Asn 645	Ile	Pro	Thr	Ala	His	Ala 650	Leu	Met	Leu	Ser	Asn 655	Lys
Asp	Ser	Ile	Thr 660	Arg	Val	Tyr	Tyr	Gly 665	Asp	Leu	Tyr	Thr	Asp 670	Asp	Gly
Gln	Tyr 675	Met	Glu	Lys	Lys	Ser	Pro 680	Tyr	His	Asp	Ala	Ile 685	Asp	Ala	Leu
Leu 690	Arg	Ala	Arg	Ile	Lys	Tyr 695	Val	Ala	Gly	Gly	Gln 700	Asp	Met	Lys	Val
Thr 705	Tyr	Met	Gly	Val	Pro 710	Arg	Glu	Ala	Asp	Lys 715	Trp	Ser	Tyr	Asn	Gly 720
Ile	Leu	Thr	Ser 725	Val	Arg	Tyr	Gly	Thr	Gly 730	Ala	Asn	Glu	Ala	Thr 735	Asp
Glu	Gly	Thr 740	Ala	Glu	Thr	Arg	Thr	Gln 745	Gly	Met	Ala	Val	Ile 750	Ala	Ser
Asn	Asn 755	Pro	Asn	Leu	Lys	Leu	Asn 760	Glu	Trp	Asp	Lys	Leu 765	Gln	Val	Asn
Met 770	Gly	Ala	Ala	His	Lys	Asn 775	Gln	Tyr	Tyr	Arg	Pro 780	Val	Leu	Leu	Thr
Thr 785	Lys	Asp	Gly	Ile	Ser 790	Arg	Tyr	Leu	Thr	Asp 795	Glu	Glu	Val	Pro	Gln 800
Ser	Leu	Trp	Lys 805	Lys	Thr	Asp	Ala	Asn	Gly 810	Ile	Leu	Thr	Phe	Asp 815	Met
Asn	Asp	Ile	Ala 820	Gly	Tyr	Ser	Asn	Val	Gln 825	Val	Ser	Gly	Tyr 830	Leu	Ala
Val	Trp 835	Val	Pro	Val	Gly	Ala	Lys 840	Ala	Asp	Gln	Asp	Ala 845	Arg	Thr	Thr
Ala 850	Ser	Lys	Lys	Lys	Asn 855	Ala	Ser	Gly	Gln	Val	Tyr 860	Glu	Ser	Ser	Ala
Ala 865	Leu	Asp	Ser	Gln	Leu 870	Ile	Tyr	Glu	Gly	Phe 875	Ser	Asn	Phe	Gln	Asp 880
Phe	Ala	Thr	Arg 885	Asp	Asp	Gln	Tyr	Thr	Asn 890	Lys	Val	Ile	Ala	Lys 895	Asn
Val	Asn 900	Leu	Phe	Lys	Glu	Trp	Gly	Val	Thr 905	Ser	Phe	Glu	Leu	Pro	Pro
Gln	Tyr 915	Val	Ser	Ser	Gln	Asp	Gly	Thr	Phe 920	Leu	Asp	Ser	Ile	Ile	Gln
Asn 930	Gly	Tyr	Ala	Phe	Glu	Asp 935	Arg	Tyr	Asp	Met	Ala 940	Met	Ser	Lys	Asn
Asn 945	Lys	Tyr	Gly	Ser	Leu 950	Lys	Asp	Leu	Leu	Asn 955	Ala	Leu	Arg	Ala	Leu 960
His	Ser	Val	Asn	Ile	Gln	Ala	Ile	Ala	Asp	Trp	Val	Pro	Asp	Gln	Ile

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965						970						975					
Tyr	Asn	Leu	Pro	Gly	Lys	Glu	Val	Val	Thr	Ala	Thr	Arg	Val	Asn	Asn		
980						985						990					
Tyr	Gly	Thr	Tyr	Arg	Glu	Gly	Ala	Glu	Ile	Lys	Glu	Lys	Leu	Tyr	Val		
995						1000						1005					
Ala	Asn	Ser	Lys	Thr	Asn	Glu	Thr	Asp	Phe	Gln	Gly	Lys	Tyr	Gly			
1010						1015						1020					
Gly	Ala	Phe	Leu	Asp	Glu	Leu	Lys	Ala	Lys	Tyr	Pro	Glu	Ile	Phe			
1025						1030						1035					
Glu	Arg	Val	Gln	Ile	Ser	Asn	Gly	Gln	Lys	Met	Thr	Thr	Asp	Glu			
1040						1045						1050					
Lys	Ile	Thr	Lys	Trp	Ser	Ala	Lys	Tyr	Phe	Asn	Gly	Thr	Asn	Ile			
1055						1060						1065					
Leu	Gly	Arg	Gly	Ala	Tyr	Tyr	Val	Leu	Lys	Asp	Trp	Ala	Ser	Asn			
1070						1075						1080					
Asp	Tyr	Leu	Thr	Asn	Arg	Asn	Gly	Glu	Ile	Val	Leu	Pro	Lys	Gln			
1085						1090						1095					
Leu	Val	Asn	Lys	Asn	Ser	Tyr	Thr	Gly	Phe	Val	Ser	Asp	Ala	Asn			
1100						1105						1110					
Gly	Thr	Lys	Phe	Tyr	Ser	Thr	Ser	Gly	Tyr	Gln	Ala	Lys	Asn	Ser			
1115						1120						1125					
Phe	Ile	Gln	Asp	Glu	Asn	Gly	Asn	Trp	Tyr	Tyr	Phe	Asp	Lys	Arg			
1130						1135						1140					
Gly	Tyr	Leu	Val	Thr	Gly	Ala	His	Glu	Ile	Asp	Gly	Lys	His	Val			
1145						1150						1155					
Tyr	Phe	Leu	Lys	Asn	Gly	Ile	Gln	Leu	Arg	Asp	Ser	Ile	Arg	Glu			
1160						1165						1170					
Asp	Glu	Asn	Gly	Asn	Gln	Tyr	Tyr	Tyr	Asp	Gln	Thr	Gly	Ala	Gln			
1175						1180						1185					
Val	Leu	Asn	Arg	Tyr	Tyr	Thr	Thr	Asp	Gly	Gln	Asn	Trp	Arg	Tyr			
1190						1195						1200					
Phe	Asp	Ala	Lys	Gly	Val	Met	Ala	Arg	Gly	Leu	Val	Lys	Ile	Gly			
1205						1210						1215					
Asp	Gly	Gln	Gln	Phe	Phe	Asp	Glu	Asn	Gly	Tyr	Gln	Val	Lys	Gly			
1220						1225						1230					
Lys	Ile	Val	Ser	Ala	Lys	Asp	Gly	Lys	Leu	Arg	Tyr	Phe	Asp	Lys			
1235						1240						1245					
Asp	Ser	Gly	Asn	Ala	Val	Ile	Asn	Arg	Phe	Ala	Gln	Gly	Asp	Asn			
1250						1255						1260					
Pro	Ser	Asp	Trp	Tyr	Tyr	Phe	Gly	Val	Glu	Phe	Ala	Lys	Leu	Thr			
1265						1270						1275					
Gly	Leu	Gln	Lys	Ile	Gly	Gln	Gln	Thr	Leu	Tyr	Phe	Asp	Gln	Asp			
1280						1285						1290					
Gly	Lys	Gln	Val	Lys	Gly	Lys	Ile	Val	Thr	Leu	Ser	Asp	Lys	Ser			
1295						1300						1305					
Ile	Arg	Tyr	Phe	Asp	Ala	Asn	Ser	Gly	Glu	Met	Ala	Val	Gly	Lys			
1310						1315						1320					
Phe	Ala	Glu	Gly	Ala	Lys	Asn	Glu	Trp	Tyr	Tyr	Phe	Asp	Lys	Thr			
1325						1330						1335					
Gly	Lys	Ala	Val	Thr	Gly	Leu	Gln	Lys	Ile	Gly	Lys	Gln	Thr	Leu			
1340						1345						1350					
Tyr	Phe	Asp	Gln	Asp	Gly	Lys	Gln	Val	Lys	Gly	Lys	Val	Val	Thr			
1355						1360						1365					

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Leu Ala Asp Lys Ser Ile Arg Tyr Phe Asp Ala Asp Ser Gly Glu
 1370 1375 1380
 Met Ala Val Gly Lys Phe Ala Glu Gly Ala Lys Asn Glu Trp Tyr
 1385 1390 1395
 Tyr Phe Asp Gln Thr Gly Lys Ala Val Thr Gly Leu Gln Lys Ile
 1400 1405 1410
 Asp Lys Gln Thr Leu Tyr Phe Asp Gln Asp Gly Lys Gln Val Lys
 1415 1420 1425
 Gly Lys Ile Val Thr Leu Ser Asp Lys Ser Ile Arg Tyr Phe Asp
 1430 1435 1440
 Ala Asn Ser Gly Glu Met Ala Thr Asn Lys Phe Val Glu Gly Ser
 1445 1450 1455
 Gln Asn Glu Trp Tyr Tyr Phe Asp Gln Ala Gly Lys Ala Val Thr
 1460 1465 1470
 Gly Leu Gln Gln Val Gly Gln Gln Thr Leu Tyr Phe Thr Gln Asp
 1475 1480 1485
 Gly Lys Gln Val Lys Gly Lys Val Val Asp Val Asn Gly Val Ser
 1490 1495 1500
 Arg Tyr Phe Asp Ala Asn Ser Gly Asp Met Ala Arg Ser Lys Trp
 1505 1510 1515
 Ile Gln Leu Glu Asp Gly Ser Trp Met Tyr Phe Asp Arg Asp Gly
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 Arg Gly Gln Asn Phe Gly Arg Asn
 1535 1540

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<211> LENGTH: 3014

<212> TYPE: DNA

<213> ORGANISM: Zea mays

<220> FEATURE:

<221> NAME/KEY: promoter

<222> LOCATION: (1)..(3014)

<223> OTHER INFORMATION: maize ubiquitin promoter

<400> SEQUENCE: 39

```

gacaaacctc tatatgtaga gtacaggagc ttttacagga ccctgctgga gccagcctta    60
gggggaaaac ttccaggcgg taggtcacat acatcagtga ggtaggagaa atgtgccaac    120
cacgtggtgt cgaccaatct acattctaata ctatatcatt atataattta tcagtttaaa    180
ctttacaaaa tctatctaaa caaatcacat ctacacccat aacattcggt aaatctaaca    240
cagtatcaaa actagcgggt caaatcgatg gataacatgt tctcccatat ccattcaaat    300
ctgatagata atattattta gatcatgtat tctctctccc ctctccctcg acgcctcttc    360
ctgccccgtg tccctgacct gtctccctca cttatgatgt tgtctctatc atcaatcgct    420
ccttttatat tgtgatcact gtccaccctt attcctactc gggattaggg atggcaatgg    480
aaaatttctc atcgaggaat agctcttcat acccatccca cgacgcagaa atttcctcgc    540
gggaataccc acgaacgttt acagaagaca tttcttcccc atccatatcc ccacgggca    600
taaatttccg acggagatca acgtccctat ttacattata attaggaaat gcatcctttg    660
ttattaataa aaacactttc acttatatat attgttagat gtaagaaatc attatgggta    720
tattaaaata aacatatattg tacaatgatt gatctcttac ccaataaatt atttgttttt    780
attattagct agtatacgaa aacatcacca cgtacaggtt tgacggattc ccacagaaac    840
agggatgaaa aatacttota catccctgtc ccgtttaccc atctgagaaa gcgggaaatc    900

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gggcatagga tccattgcca aagatcgtag ggctataacc taagcgttgc aacgaagcga 960
agcagacggt ggagacgttg acgcaaagca atgaacttga acggcatctc tctcgctggc 1020
cctggccttc tcgaaggctc tgcgtgggtc cttgcgcagt tgcgccgcag cgggctggca 1080
gcatccggaa attgctcttt gcgtggcgga gcagacacta aggtactatt ttacgttcta 1140
tttagttgga ctgtggcggg aaactatgaa aaaaactatt gcagactatg agctattaaa 1200
aagctaaaaa ttattttagt taaaccacta aaaaccatta aaaattcttt gatatatatt 1260
ttcacagttt tataaaaaat ccactaaaaa cagggtcaaat aagctttcaa ttttacta 1320
cgaaaaagtc agctttttaa aaaaactgct taaatccagt ccttttagtt aatttttatc 1380
ttttaggaaa caaaagccaa aactaaaacc aaaccaaacc tacctttaa accgatctaa 1440
taggaacgcg gtgtttggaa caactagata ttaattttag aggttagacc gccacgaaag 1500
cgtcactgca cacggcattc cctcccca cgttatcgt cgcaccataa ataaccatcc 1560
tctctcgcc tttcccaca tctcatctc gtctgtgttc ttgggcgtac gcggacacag 1620
ccccgatccg aatcgctgc cttgcgagcc tcgccgatcc ccaactcccc tcccctcgt 1680
tcaaggtaac tgcatcatc catcctcccg ctccactct cccttcacct cctctgcttg 1740
ctaggtatac gaacatacga tttattacgg gttatatggg ggcttcgatt ccagatctg 1800
gcgatctatt atcgtagctc cgagtcctcg atctagtaat tgtgggatat gcttgtaaga 1860
ggctctgaga tgggttgggt tgggttgggt cgctgtgacg attccaacag cctcgtttct 1920
taggttggga tcttctcgtg gtttcccttt taattaaata agtacctgat gcagaatggt 1980
gcgtcctatt agatggaacc ttgatcttga tgcataaac cttgatcttg ttcgctgtga 2040
tgattccaac aggctcgttt cttaggcctg ttcgtctggt tcgtcagatc agtttcgttg 2100
cttttgccct cgttgtaagg tccatccaga tcggagtaga atcgaatgat ttattatacg 2160
gtagctgctg gtctcattag atttgatct gcattgggtg aacatatgta ttcataatta 2220
atatggtgta tacgtactag tttgctggtc ttattttttt agcctgattg cttctgcctt 2280
tctggcaacg cctgatccac gcgttagcta gagggtgatt tagttccttg tttacgcggc 2340
cacacctgcc gcctagaaaa gctgcagcga gaactctaata taaatttga tctacatgtg 2400
ctagcatata tgtttgtaat taatatgatg gatgaatatg tgcttcagag ttgagttcct 2460
gttgatgctg tagttctgcc tgaattgttg aggtgtagc ttctgcctga ttaaaatgca 2520
ccgtgcctat ctgttaaaact ctagggtgtg tgatttagcc ggtgacggtg gtttaatatg 2580
tgtaatttca ctgcttatag taatgcaatt cacccttgct tgaacatgca ttgtcttggt 2640
gctttgttct atacacatgc ttagctatta tctgatgagc atgcactgtt ttgttctggt 2700
tgatatgcat gctcagaaat atgtagatgt gtggctcctg ctcggttggt ctttateatc 2760
cacctgttga acatgcatgt tctgtcgct tatctttatt atatattacc ttcgttctcg 2820
aatatttgc gcccgctagt tcatttttga actaaaccgt gacaaataaa atagaacgta 2880
gggagtggca tcattgctgt actgtacctt acggtggcaa ctacatcttg agcacgcata 2940
tatcttatag tgttcctttt ctttctctcc ttggtctact gttatatgct tacctttttt 3000
tggtttcctt gcag 3014

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<210> SEQ ID NO 40

<211> LENGTH: 68

<212> TYPE: DNA

<213> ORGANISM: tobacco mosaic virus

<220> FEATURE:

<221> NAME/KEY: enhancer

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<222> LOCATION: (1)..(68)
 <223> OTHER INFORMATION: TMV enhancer

<400> SEQUENCE: 40

gtattttttac aacaattacc aacaacaaca aacaacaac aacattacaa ttactattta 60
 cataaaacc 68

<210> SEQ ID NO 41
 <211> LENGTH: 195
 <212> TYPE: DNA
 <213> ORGANISM: Cyanophora paradoxa
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(195)
 <223> OTHER INFORMATION: chloroplast targeting sequence FNR

<400> SEQUENCE: 41

atg gct ttc gtt gcc agc gtg cca gtt ttc gct aac gct tcc gcc ctt 48
 Met Ala Phe Val Ala Ser Val Pro Val Phe Ala Asn Ala Ser Gly Leu
 1 5 10 15
 aaa act gaa gcc aag gtg tgc cag aag cct gcc ttg aag aat tca ttt 96
 Lys Thr Glu Ala Lys Val Cys Gln Lys Pro Ala Leu Lys Asn Ser Phe
 20 25 30
 ttc agg gcc gag gaa gtc aca tct aga tct ttt ttt gcc tcc caa gca 144
 Phe Arg Gly Glu Glu Val Thr Ser Arg Ser Phe Phe Ala Ser Gln Ala
 35 40 45
 gtg tcc gct aaa cca gca aca acc gcc gag gtt gat act acc att agg 192
 Val Ser Ala Lys Pro Ala Thr Thr Gly Glu Val Asp Thr Thr Ile Arg
 50 55 60
 gca 195
 Ala
 65

<210> SEQ ID NO 42
 <211> LENGTH: 65
 <212> TYPE: PRT
 <213> ORGANISM: Cyanophora paradoxa

<400> SEQUENCE: 42

Met Ala Phe Val Ala Ser Val Pro Val Phe Ala Asn Ala Ser Gly Leu
 1 5 10 15
 Lys Thr Glu Ala Lys Val Cys Gln Lys Pro Ala Leu Lys Asn Ser Phe
 20 25 30
 Phe Arg Gly Glu Glu Val Thr Ser Arg Ser Phe Phe Ala Ser Gln Ala
 35 40 45
 Val Ser Ala Lys Pro Ala Thr Thr Gly Glu Val Asp Thr Thr Ile Arg
 50 55 60
 Ala
 65

<210> SEQ ID NO 43
 <211> LENGTH: 1590
 <212> TYPE: DNA
 <213> ORGANISM: unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic gene
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1590)
 <223> OTHER INFORMATION: maize codon optimized alpha-1,5-glucosidase
 from Thermus thermophilus

<400> SEQUENCE: 43

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atg tcc tgg tgg cag cgc gcc gtg atc tac cag gtg tac ccg cgc agc	48
Met Ser Trp Trp Gln Arg Ala Val Ile Tyr Gln Val Tyr Pro Arg Ser	
1 5 10 15	
ttc cag gac acc aac ggc gac gga gtg ggc gac ctc gag ggc atc agg	96
Phe Gln Asp Thr Asn Gly Asp Gly Val Gly Asp Leu Glu Gly Ile Arg	
20 25 30	
cgc agg ctg ccg tac ctg aag tcc ctg gga gtg gat gcc ctg tgg ctg	144
Arg Arg Leu Pro Tyr Leu Lys Ser Leu Gly Val Asp Ala Leu Trp Leu	
35 40 45	
tcc ccg ttc tac aag agc ccg atg aag gac ttt gga tac gat gtg gct	192
Ser Pro Phe Tyr Lys Ser Pro Met Lys Asp Phe Gly Tyr Asp Val Ala	
50 55 60	
gac tac tgc gac gtg gac ccg gtg ttc ggc acc ctc cag gac ttc gac	240
Asp Tyr Cys Asp Val Asp Pro Val Phe Gly Thr Leu Gln Asp Phe Asp	
65 70 75 80	
cgc ctg ctc gag gaa gct cac gct ctt gga ctg aag gtt ctc gtc gat	288
Arg Leu Leu Glu Ala His Ala Leu Gly Leu Lys Val Leu Val Asp	
85 90 95	
ctc gtg ccg aac cac acc agc agc gag cac ccg tgg ttc ctc gag agc	336
Leu Val Pro Asn His Thr Ser Ser Glu His Pro Trp Phe Leu Glu Ser	
100 105 110	
cgc gcc agc agg aac agc ccg aag cgc gac tgg tac atc tgg aag gac	384
Arg Ala Ser Arg Asn Ser Pro Lys Arg Asp Trp Tyr Ile Trp Lys Asp	
115 120 125	
cca gcc cca gat ggc ggc cca ccg aac aac tgg cag agc ttc ttc gga	432
Pro Ala Pro Asp Gly Gly Pro Pro Asn Asn Trp Gln Ser Phe Phe Gly	
130 135 140	
ggg cca gct tgg act ctg gac gag gct aca ggc caa tat tac ctc cac	480
Gly Pro Ala Trp Thr Leu Asp Glu Ala Thr Gly Gln Tyr Tyr Leu His	
145 150 155 160	
ctg ttc ctc cca gag cag ccg gac ctg aac tgg cgc aac ccc gaa gtg	528
Leu Phe Leu Pro Glu Gln Pro Asp Leu Asn Trp Arg Asn Pro Glu Val	
165 170 175	
cgc gag gcc atc aag gaa gtg atg cgc ttc tgg ctc aga cgc ggt gtc	576
Arg Glu Ala Ile Lys Glu Val Met Arg Phe Trp Leu Arg Arg Gly Val	
180 185 190	
gat ggc ttc agg gtg gac gtg ctg tgg ctg ctg ggc aag gac ccg ctg	624
Asp Gly Phe Arg Val Asp Val Leu Trp Leu Leu Gly Lys Asp Pro Leu	
195 200 205	
ttc agg gac gag ccg ggc agc cca ctg tgg agg cca ggc ctg cca gac	672
Phe Arg Asp Glu Pro Gly Ser Pro Leu Trp Arg Pro Gly Leu Pro Asp	
210 215 220	
agg gcc agg cac gag cac ctg tac acc gag gac cag ccc gag act tac	720
Arg Ala Arg His Glu His Leu Tyr Thr Glu Asp Gln Pro Glu Thr Tyr	
225 230 235 240	
gcc tat gtg cgc gag atg cgc cag gtg ctg gac gag ttc agc gag cca	768
Ala Tyr Val Arg Glu Met Arg Gln Val Leu Asp Glu Phe Ser Glu Pro	
245 250 255	
ggc cgc gag agg gtg atg gtg ggc gag atc tac ctc cca ctc cca cgg	816
Gly Arg Glu Arg Val Met Val Gly Glu Ile Tyr Leu Pro Leu Pro Arg	
260 265 270	
ctt gtg agg tac tac gcc gct ggc tgc cac ctc ccg ttc aac ttc agc	864
Leu Val Arg Tyr Tyr Ala Ala Gly Cys His Leu Pro Phe Asn Phe Ser	
275 280 285	
ctg gtg acg gaa ggt ctc agc gac tgg cgc cca gag aac ctg gcc cgc	912
Leu Val Thr Glu Gly Leu Ser Asp Trp Arg Pro Glu Asn Leu Ala Arg	
290 295 300	
atc gtg gag act tac gag ggg ctc ctg tct aga tgg gat tgg cca aac	960
Ile Val Glu Thr Tyr Glu Gly Leu Leu Ser Arg Trp Asp Trp Pro Asn	
305 310 315 320	

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tgg gtg ctg ggc aac cac gat caa cct cgc ctc gct tcc cgc ctc ggc	1008
Trp Val Leu Gly Asn His Asp Gln Pro Arg Leu Ala Ser Arg Leu Gly	
325 330 335	
gag cca cag gcc agg gtg gcc gcc atg ctg ctg ttc acc ctg agg ggc	1056
Glu Pro Gln Ala Arg Val Ala Ala Met Leu Leu Phe Thr Leu Arg Gly	
340 345 350	
acc ccg acc tgg tac tac ggg gat gaa ctc gct ctc ccg aac ggg ctc	1104
Thr Pro Thr Trp Tyr Tyr Gly Asp Glu Leu Ala Leu Pro Asn Gly Leu	
355 360 365	
att cca cca gag aag gtc caa gac cca gca gca ctg agg cag agg gac	1152
Ile Pro Pro Glu Lys Val Gln Asp Pro Ala Ala Leu Arg Gln Arg Asp	
370 375 380	
cgc gag cca acc gcc tac cac acc ctg ggc cgc gat cca gag ccg act	1200
Arg Glu Pro Thr Ala Tyr His Thr Leu Gly Arg Asp Pro Glu Arg Thr	
385 390 395 400	
cca atg cct tgg gac gcc agc cca tac ggc ggc ttc agc acc gtg gag	1248
Pro Met Pro Trp Asp Ala Ser Pro Tyr Gly Gly Phe Ser Thr Val Glu	
405 410 415	
cct tgg ctc cca ctg aac ccg gac tac cgc acc agg aac gtc gct gct	1296
Pro Trp Leu Pro Leu Asn Pro Asp Tyr Arg Thr Arg Asn Val Ala Ala	
420 425 430	
caa gag aag gac cca ccg tcc atg ctc cac ctg gtc aag cgc ctg att	1344
Gln Glu Lys Asp Pro Arg Ser Met Leu His Leu Val Lys Arg Leu Ile	
435 440 445	
gct ctc cgc aag gac ccg gac ctc ctg tac ggc gcc tac cgc acc tac	1392
Ala Leu Arg Lys Asp Pro Asp Leu Leu Tyr Gly Ala Tyr Arg Thr Tyr	
450 455 460	
cgc gct ccg gag gga gtc tac gcc tac ctc cgc ggg gag ggc tgg ctc	1440
Arg Ala Arg Glu Gly Val Tyr Ala Tyr Leu Arg Gly Glu Gly Trp Leu	
465 470 475 480	
gtg gcc ctg aac ctg acc gag aag gaa aag gcc ctc gag ctg cca agg	1488
Val Ala Leu Asn Leu Thr Glu Lys Glu Lys Ala Leu Glu Leu Pro Arg	
485 490 495	
ggc ggc agg gtc gtc ctg tcc acc cac ctg gac aga gag gaa aga gtc	1536
Gly Gly Arg Val Val Leu Ser Thr His Leu Asp Arg Glu Glu Arg Val	
500 505 510	
ggg gaa agg ctg ttc ctc aga cct gat gaa ggc gtc gct gtc aga ctg	1584
Gly Glu Arg Leu Phe Leu Arg Pro Asp Glu Gly Val Ala Val Arg Leu	
515 520 525	
gac tga	1590
Asp	

<210> SEQ ID NO 44

<211> LENGTH: 529

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 44

Met Ser Trp Trp Gln Arg Ala Val Ile Tyr Gln Val Tyr Pro Arg Ser
1 5 10 15

Phe Gln Asp Thr Asn Gly Asp Gly Val Gly Asp Leu Glu Gly Ile Arg
20 25 30

Arg Arg Leu Pro Tyr Leu Lys Ser Leu Gly Val Asp Ala Leu Trp Leu
35 40 45

Ser Pro Phe Tyr Lys Ser Pro Met Lys Asp Phe Gly Tyr Asp Val Ala
50 55 60

Asp Tyr Cys Asp Val Asp Pro Val Phe Gly Thr Leu Gln Asp Phe Asp

65	70								75							80			
Arg	Leu	Leu	Glu	Glu	Ala	His	Ala	Leu	Gly	Leu	Lys	Val	Leu	Val	Asp				
85									90					95					
Leu	Val	Pro	Asn	His	Thr	Ser	Ser	Glu	His	Pro	Trp	Phe	Leu	Glu	Ser				
100								105					110						
Arg	Ala	Ser	Arg	Asn	Ser	Pro	Lys	Arg	Asp	Trp	Tyr	Ile	Trp	Lys	Asp				
115							120					125							
Pro	Ala	Pro	Asp	Gly	Gly	Pro	Pro	Asn	Asn	Trp	Gln	Ser	Phe	Phe	Gly				
130						135				140									
Gly	Pro	Ala	Trp	Thr	Leu	Asp	Glu	Ala	Thr	Gly	Gln	Tyr	Tyr	Leu	His				
145					150					155					160				
Leu	Phe	Leu	Pro	Glu	Gln	Pro	Asp	Leu	Asn	Trp	Arg	Asn	Pro	Glu	Val				
165								170					175						
Arg	Glu	Ala	Ile	Lys	Glu	Val	Met	Arg	Phe	Trp	Leu	Arg	Arg	Gly	Val				
180								185					190						
Asp	Gly	Phe	Arg	Val	Asp	Val	Leu	Trp	Leu	Leu	Gly	Lys	Asp	Pro	Leu				
195							200					205							
Phe	Arg	Asp	Glu	Pro	Gly	Ser	Pro	Leu	Trp	Arg	Pro	Gly	Leu	Pro	Asp				
210						215					220								
Arg	Ala	Arg	His	Glu	His	Leu	Tyr	Thr	Glu	Asp	Gln	Pro	Glu	Thr	Tyr				
225					230					235					240				
Ala	Tyr	Val	Arg	Glu	Met	Arg	Gln	Val	Leu	Asp	Glu	Phe	Ser	Glu	Pro				
245								250					255						
Gly	Arg	Glu	Arg	Val	Met	Val	Gly	Glu	Ile	Tyr	Leu	Pro	Leu	Pro	Arg				
260							265					270							
Leu	Val	Arg	Tyr	Tyr	Ala	Ala	Gly	Cys	His	Leu	Pro	Phe	Asn	Phe	Ser				
275							280					285							
Leu	Val	Thr	Glu	Gly	Leu	Ser	Asp	Trp	Arg	Pro	Glu	Asn	Leu	Ala	Arg				
290						295					300								
Ile	Val	Glu	Thr	Tyr	Glu	Gly	Leu	Leu	Ser	Arg	Trp	Asp	Trp	Pro	Asn				
305					310					315					320				
Trp	Val	Leu	Gly	Asn	His	Asp	Gln	Pro	Arg	Leu	Ala	Ser	Arg	Leu	Gly				
325								330					335						
Glu	Pro	Gln	Ala	Arg	Val	Ala	Ala	Met	Leu	Leu	Phe	Thr	Leu	Arg	Gly				
340							345					350							
Thr	Pro	Thr	Trp	Tyr	Tyr	Gly	Asp	Glu	Leu	Ala	Leu	Pro	Asn	Gly	Leu				
355							360					365							
Ile	Pro	Pro	Glu	Lys	Val	Gln	Asp	Pro	Ala	Ala	Leu	Arg	Gln	Arg	Asp				
370						375					380								
Arg	Glu	Pro	Thr	Ala	Tyr	His	Thr	Leu	Gly	Arg	Asp	Pro	Glu	Arg	Thr				
385					390					395					400				
Pro	Met	Pro	Trp	Asp	Ala	Ser	Pro	Tyr	Gly	Gly	Phe	Ser	Thr	Val	Glu				
405									410				415						
Pro	Trp	Leu	Pro	Leu	Asn	Pro	Asp	Tyr	Arg	Thr	Arg	Asn	Val	Ala	Ala				
420							425					430							

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Gly Gly Arg Val Val Leu Ser Thr His Leu Asp Arg Glu Glu Arg Val
500 505 510

Gly Glu Arg Leu Phe Leu Arg Pro Asp Glu Gly Val Ala Val Arg Leu
515 520 525

Asp

<210> SEQ ID NO 45
<211> LENGTH: 1001
<212> TYPE: DNA
<213> ORGANISM: Zea maize
<220> FEATURE:
<221> NAME/KEY: terminator
<222> LOCATION: (1)..(1001)
<223> OTHER INFORMATION: maize ubi terminator

<400> SEQUENCE: 45

```
gccatcagtc gttgaagctg ctgctgtatc tgggttatct agtgtctctg ccattgccca      60
aggatggtgc tgtctttcaa agtatttgta tggtttgtgt cgtgagtcgt gactgagctg      120
gtttcatgga ccagtttgtt tctcgttacc caaaactatc gtgcgacgcg atatggctta      180
atcatgaata aatgttgttt gaatttaaac tattcgctga atattgttgt tttttgtcat      240
gtcagttaat gttactaaat tggttgcctt ctaatttttg tttactgggtg tttgtcgcac      300
cttatctttt tactgtatgt ttacttcagg ttctggcagt ctcatttttt gtgactagtt      360
aaaacttaca gctaaaaaaa tgcagttttt cattttcatt tgaagtttga ttagagctat      420
tgatacccg accatcaggt taggttagtt gtgcatagaa tcataaatat taatcatggt      480
ttctatgaat taagtcaaac ttgaaagtct ggctgaatat agtttctatg aatcatattg      540
atatacatgt ttgattattt gttttgctat tagctattta ctttggtgaa tctatatagg      600
cttatgcaga accttttttt ttgttctata tatccatata ctagtactca gtagctctat      660
gttttctgga gactagtggc ttgctttttc gtatgtctaa ttttttgctt gaccattgca      720
aaacaaaaat tacctagtgt aatctctttt tataataatc ttgtaatgcg tctacctata      780
gggtcaaagta ggtttttgtt ggaaccctta gagctaactg ttagctagtt gataaattat      840
tagctgagtt aagctagcta atgaactagt tttgatatta gctgaggatg tttgaaacct      900
aataattatt ttttattagc taactatact aaattttagt agagagattc caaacaggag      960
ttaacatggg atcagattgg ctatgcgttt gcaatcccat a                          1001
```

<210> SEQ ID NO 46
<211> LENGTH: 1590
<212> TYPE: DNA
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: synthetic gene
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1590)
<223> OTHER INFORMATION: dicot optimized alpha-1,5-glucosidase HB8

<400> SEQUENCE: 46

```
atg tct tgg tgg caa agg gct gtt atc tac caa gtt tac cca aga tcc      48
Met Ser Trp Trp Gln Arg Ala Val Ile Tyr Gln Val Tyr Pro Arg Ser
1 5 10 15

ttc cag gat aca aac ggt gat ggt gtt gga gat ctt gag gga att aga      96
Phe Gln Asp Thr Asn Gly Asp Gly Val Gly Asp Leu Glu Gly Ile Arg
20 25 30

aga agg ctt cca tac ctt aag tct ctt gga gtt gat gct ctt tgg ctt      144
Arg Arg Leu Pro Tyr Leu Lys Ser Leu Gly Val Asp Ala Leu Trp Leu
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35	40	45	
tcc cca ttc tac aag tcc cca atg aag gat ttc gga tac gat gtt gct			192
Ser Pro Phe Tyr Lys Ser Ser Pro Met Lys Asp Phe Gly Tyr Asp Val Ala			
50	55	60	
gat tac tgt gat gtt gat cca gtg ttc gga act ctt cag gat ttc gat			240
Asp Tyr Cys Asp Val Asp Pro Val Phe Gly Thr Leu Gln Asp Phe Asp			
65	70	75	80
agg ctt ctt gaa gag gct cat gct ctt gga ctt aag gtg ttg gtt gat			288
Arg Leu Leu Glu Glu Ala His Ala Leu Gly Leu Lys Val Leu Val Asp			
85	90	95	
ctt gtt cca aac cac act tct tca gag cat cct tgg ttt ctt gaa tct			336
Leu Val Pro Asn His Thr Ser Ser Glu His Pro Trp Phe Leu Glu Ser			
100	105	110	
agg gct tct agg aat tct cca aag agg gat tgg tac atc tgg aaa gat			384
Arg Ala Ser Arg Asn Ser Pro Lys Arg Asp Trp Tyr Ile Trp Lys Asp			
115	120	125	
cca gct cca gat ggt gga cca cca aat aac tgg cag tct ttc ttt ggt			432
Pro Ala Pro Asp Gly Gly Pro Pro Asn Asn Trp Gln Ser Phe Phe Gly			
130	135	140	
ggt cct gct tgg act ctt gat gaa gct act gga cag tac tac ctt cac			480
Gly Pro Ala Trp Thr Leu Asp Glu Ala Thr Gly Gln Tyr Tyr Leu His			
145	150	155	160
ttg ttc ctt cca gag cag cca gat ctt aat tgg agg aac cca gaa gtt			528
Leu Phe Leu Pro Glu Gln Pro Asp Leu Asn Trp Arg Asn Pro Glu Val			
165	170	175	
aga gag gct atc aaa gaa gtt atg agg ttc tgg ctt aga aga ggt gtt			576
Arg Glu Ala Ile Lys Glu Val Met Arg Phe Trp Leu Arg Arg Gly Val			
180	185	190	
gat gga ttc aga gtg gat gtg ctt tgg ctt ctt gga aaa gat cca ctc			624
Asp Gly Phe Arg Val Asp Val Leu Trp Leu Leu Gly Lys Asp Pro Leu			
195	200	205	
ttt agg gat gaa cca gga tct cca ctt tgg agg cca gga ctt cca gat			672
Phe Arg Asp Glu Pro Gly Ser Pro Leu Trp Arg Pro Gly Leu Pro Asp			
210	215	220	
aga gct agg cat gag cat ctt tac act gag gat cag cca gag act tat			720
Arg Ala Arg His Glu His Leu Tyr Thr Glu Asp Gln Pro Glu Thr Tyr			
225	230	235	240
gct tat gtg aga gag atg agg caa gtt ctt gat gag ttt tct gag cca			768
Ala Tyr Val Arg Glu Met Arg Gln Val Leu Asp Glu Phe Ser Glu Pro			
245	250	255	
gga cgt gaa aga gtt atg gtt gga gag atc tac ctt cca ctt cca agg			816
Gly Arg Glu Arg Val Met Val Gly Glu Ile Tyr Leu Pro Leu Pro Arg			
260	265	270	
ctt gtt agg tat tat gct gct gga tgc cat ctt cca ttc aac ttc tca			864
Leu Val Arg Tyr Tyr Ala Ala Gly Cys His Leu Pro Phe Asn Phe Ser			
275	280	285	
ctt gtg act gag gga ctt tct gat tgg agg cca gaa aac ctt gct aga			912
Leu Val Thr Glu Gly Leu Ser Asp Trp Arg Pro Glu Asn Leu Ala Arg			
290	295	300	
atc gtg gaa act tac gag gga ctt ctt tct aga tgg gat tgg cca aat			960
Ile Val Glu Thr Tyr Glu Gly Leu Leu Ser Arg Trp Asp Trp Pro Asn			
305	310	315	320
tgg gtt ttg gga aac cat gat cag cct aga ctt gct tct aga ctt gga			1008
Trp Val Leu Gly Asn His Asp Gln Pro Arg Leu Ala Ser Arg Leu Gly			
325	330	335	
gaa cca caa gct aga gtt gct gct atg ctt ttg ttc act ctc aga gga			1056
Glu Pro Gln Ala Arg Val Ala Ala Met Leu Leu Phe Thr Leu Arg Gly			
340	345	350	
act cca act tgg tat tac ggt gat gag ctt gct ttg cca aac gga ctt			1104

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Thr	Pro	Thr	Trp	Tyr	Tyr	Gly	Asp	Glu	Leu	Ala	Leu	Pro	Asn	Gly	Leu	
		355					360					365				
att	cca	cca	gag	aag	gtt	caa	gat	cca	gct	gct	ctt	aga	caa	aga	gat	1152
Ile	Pro	Pro	Glu	Lys	Val	Gln	Asp	Pro	Ala	Ala	Leu	Arg	Gln	Arg	Asp	
		370				375					380					
aga	gag	cca	act	gct	tac	cat	act	ctt	ggg	aga	gat	cca	gaa	aga	act	1200
Arg	Glu	Pro	Thr	Ala	Tyr	His	Thr	Leu	Gly	Arg	Asp	Pro	Glu	Arg	Thr	
		385			390					395					400	
cca	atg	cct	tg	gat	gct	tct	cca	tat	ggg	gga	ttc	tct	act	gtt	gaa	1248
Pro	Met	Pro	Trp	Asp	Ala	Ser	Pro	Tyr	Gly	Gly	Phe	Ser	Thr	Val	Glu	
				405					410					415		
cct	tg	ctt	cca	ctt	aat	cca	gat	tac	cgt	act	agg	aat	gtt	gct	gct	1296
Pro	Trp	Leu	Pro	Leu	Asn	Pro	Asp	Tyr	Arg	Thr	Arg	Asn	Val	Ala	Ala	
			420					425					430			
caa	gag	aaa	gat	cca	aga	tct	atg	ctt	cac	ctt	gtg	aag	agg	ctt	att	1344
Gln	Glu	Lys	Asp	Pro	Arg	Ser	Met	Leu	His	Leu	Val	Lys	Arg	Leu	Ile	
		435					440					445				
gct	ctc	agg	aaa	gat	cct	gat	ctt	ctc	tat	ggg	gct	tac	cgt	act	tat	1392
Ala	Leu	Arg	Lys	Asp	Pro	Asp	Leu	Leu	Tyr	Gly	Ala	Tyr	Arg	Thr	Tyr	
		450				455					460					
cgt	gct	aga	gag	ggc	gtt	tac	gct	tat	ctt	agg	ggg	gaa	gga	tg	ctt	1440
Arg	Ala	Arg	Glu	Gly	Val	Tyr	Ala	Tyr	Leu	Arg	Gly	Glu	Gly	Trp	Leu	
		465			470					475					480	
gtt	gct	ctt	aac	ctc	act	gag	aaa	gag	aag	gct	ctt	gaa	ctt	cca	aga	1488
Val	Ala	Leu	Asn	Leu	Thr	Glu	Lys	Glu	Lys	Ala	Leu	Glu	Leu	Pro	Arg	
			485						490					495		
ggg	gga	aga	gtt	gtg	ctt	tct	act	cac	ctt	gat	agg	gaa	gaa	aga	gtt	1536
Gly	Gly	Arg	Val	Val	Leu	Ser	Thr	His	Leu	Asp	Arg	Glu	Glu	Arg	Val	
			500					505					510			
gga	gag	agg	ctt	ttt	ctt	agg	cct	gat	gaa	ggg	gtt	gct	gtt	aga	ctt	1584
Gly	Glu	Arg	Leu	Phe	Leu	Arg	Pro	Asp	Glu	Gly	Val	Ala	Val	Arg	Leu	
		515					520					525				
gat	taa															1590
Asp																

<210> SEQ ID NO 47

<211> LENGTH: 529

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 47

Met	Ser	Trp	Trp	Gln	Arg	Ala	Val	Ile	Tyr	Gln	Val	Tyr	Pro	Arg	Ser	
1			5						10					15		
Phe	Gln	Asp	Thr	Asn	Gly	Asp	Gly	Val	Gly	Asp	Leu	Glu	Gly	Ile	Arg	
		20					25						30			
Arg	Arg	Leu	Pro	Tyr	Leu	Lys	Ser	Leu	Gly	Val	Asp	Ala	Leu	Trp	Leu	
		35					40					45				
Ser	Pro	Phe	Tyr	Lys	Ser	Pro	Met	Lys	Asp	Phe	Gly	Tyr	Asp	Val	Ala	
		50				55					60					
Asp	Tyr	Cys	Asp	Val	Asp	Pro	Val	Phe	Gly	Thr	Leu	Gln	Asp	Phe	Asp	
		65			70				75					80		
Arg	Leu	Leu	Glu	Glu	Ala	His	Ala	Leu	Gly	Leu	Lys	Val	Leu	Val	Asp	
			85					90						95		
Leu	Val	Pro	Asn	His	Thr	Ser	Ser	Glu	His	Pro	Trp	Phe	Leu	Glu	Ser	
			100					105					110			
Arg	Ala	Ser	Arg	Asn	Ser	Pro	Lys	Arg	Asp	Trp	Tyr	Ile	Trp	Lys	Asp	
		115					120					125				

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Pro	Ala	Pro	Asp	Gly	Gly	Pro	Pro	Asn	Asn	Trp	Gln	Ser	Phe	Phe	Gly
130						135					140				
Gly	Pro	Ala	Trp	Thr	Leu	Asp	Glu	Ala	Thr	Gly	Gln	Tyr	Tyr	Leu	His
145					150					155					160
Leu	Phe	Leu	Pro	Glu	Gln	Pro	Asp	Leu	Asn	Trp	Arg	Asn	Pro	Glu	Val
				165					170					175	
Arg	Glu	Ala	Ile	Lys	Glu	Val	Met	Arg	Phe	Trp	Leu	Arg	Arg	Gly	Val
			180					185					190		
Asp	Gly	Phe	Arg	Val	Asp	Val	Leu	Trp	Leu	Leu	Gly	Lys	Asp	Pro	Leu
		195					200					205			
Phe	Arg	Asp	Glu	Pro	Gly	Ser	Pro	Leu	Trp	Arg	Pro	Gly	Leu	Pro	Asp
	210					215					220				
Arg	Ala	Arg	His	Glu	His	Leu	Tyr	Thr	Glu	Asp	Gln	Pro	Glu	Thr	Tyr
225					230					235					240
Ala	Tyr	Val	Arg	Glu	Met	Arg	Gln	Val	Leu	Asp	Glu	Phe	Ser	Glu	Pro
				245					250					255	
Gly	Arg	Glu	Arg	Val	Met	Val	Gly	Glu	Ile	Tyr	Leu	Pro	Leu	Pro	Arg
		260						265					270		
Leu	Val	Arg	Tyr	Tyr	Ala	Ala	Gly	Cys	His	Leu	Pro	Phe	Asn	Phe	Ser
		275					280					285			
Leu	Val	Thr	Glu	Gly	Leu	Ser	Asp	Trp	Arg	Pro	Glu	Asn	Leu	Ala	Arg
		290				295					300				
Ile	Val	Glu	Thr	Tyr	Glu	Gly	Leu	Leu	Ser	Arg	Trp	Asp	Trp	Pro	Asn
305					310					315					320
Trp	Val	Leu	Gly	Asn	His	Asp	Gln	Pro	Arg	Leu	Ala	Ser	Arg	Leu	Gly
				325					330					335	
Glu	Pro	Gln	Ala	Arg	Val	Ala	Ala	Met	Leu	Leu	Phe	Thr	Leu	Arg	Gly
			340					345					350		
Thr	Pro	Thr	Trp	Tyr	Tyr	Gly	Asp	Glu	Leu	Ala	Leu	Pro	Asn	Gly	Leu
		355				360						365			
Ile	Pro	Pro	Glu	Lys	Val	Gln	Asp	Pro	Ala	Ala	Leu	Arg	Gln	Arg	Asp
	370					375					380				
Arg	Glu	Pro	Thr	Ala	Tyr	His	Thr	Leu	Gly	Arg	Asp	Pro	Glu	Arg	Thr
385					390					395					400
Pro	Met	Pro	Trp	Asp	Ala	Ser	Pro	Tyr	Gly	Gly	Phe	Ser	Thr	Val	Glu
			405						410					415	
Pro	Trp	Leu	Pro	Leu	Asn	Pro	Asp	Tyr	Arg	Thr	Arg	Asn	Val	Ala	Ala
		420						425					430		
Gln	Glu	Lys	Asp	Pro	Arg	Ser	Met	Leu	His	Leu	Val	Lys	Arg	Leu	Ile
		435					440					445			
Ala	Leu	Arg	Lys	Asp	Pro	Asp	Leu	Leu	Tyr	Gly	Ala	Tyr	Arg	Thr	Tyr
	450					455					460				
Arg	Ala	Arg	Glu	Gly	Val	Tyr	Ala	Tyr	Leu	Arg	Gly	Glu	Gly	Trp	Leu
465					470					475					480
Val	Ala	Leu	Asn	Leu	Thr	Glu	Lys	Glu	Lys	Ala	Leu	Glu	Leu	Pro	Arg
				485					490					495	
Gly	Gly	Arg	Val	Val	Leu	Ser	Thr	His	Leu	Asp	Arg	Glu	Glu	Arg	Val
			500					505					510		
Gly	Glu	Arg	Leu	Phe	Leu	Arg	Pro	Asp	Glu	Gly	Val	Ala	Val	Arg	Leu
	515						520					525			

Asp

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<210> SEQ ID NO 48
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: synthetic sequence
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: gamma zein signal sequence like

<400> SEQUENCE: 48

atg ggc cgc gtg ctg ctc gtg gcc ctg gcc ctg ctc gct ctc gcc gcc      48
Met Gly Arg Val Leu Leu Val Ala Leu Ala Leu Leu Ala Leu Ala Ala
1           5           10          15

agc gct acc tct      60
Ser Ala Thr Ser
20

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 49

Met Gly Arg Val Leu Leu Val Ala Leu Ala Leu Leu Ala Leu Ala Ala
1           5           10          15

Ser Ala Thr Ser
20

<210> SEQ ID NO 50
<211> LENGTH: 1761
<212> TYPE: DNA
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: synthetic gene
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1761)
<223> OTHER INFORMATION: maize optimized alpha-1,1-glucosidase B.
SAM1606

<400> SEQUENCE: 50

atg agc acc gcc ctg acc cag acc agc acc aac agc cag cag agc ccg      48
Met Ser Thr Ala Leu Thr Gln Thr Ser Thr Asn Ser Gln Gln Ser Pro
1           5           10          15

atc agg cgc gcg tgg tgg aag gaa gct gtt gtc tac cag atc tac ccg      96
Ile Arg Arg Ala Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro
20          25          30

cgc agc ttc atg gac agc aac ggc gac ggc atc ggc gac ctg agg ggc      144
Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Ile Gly Asp Leu Arg Gly
35          40          45

atc ctg agc aag ctg gac tac ctg aag ctg ctg ggc gtg gac gtg ctg      192
Ile Leu Ser Lys Leu Asp Tyr Leu Lys Leu Leu Gly Val Asp Val Leu
50          55          60

tgg ctg aac ccg atc tac gac agc ccg aac gac gac atg ggc tac gac      240
Trp Leu Asn Pro Ile Tyr Asp Ser Pro Asn Asp Asp Met Gly Tyr Asp
65          70          75          80

atc cgc gac tac tac aag atc atg gaa gaa ttc ggc act atg gaa gat      288
Ile Arg Asp Tyr Tyr Lys Ile Met Glu Glu Phe Gly Thr Met Glu Asp
85          90          95

ttc gag gaa ctg ctg cgc gag gtg cac gcc agg ggc atg aag ctg gtg      336
Phe Glu Glu Leu Leu Arg Glu Val His Ala Arg Gly Met Lys Leu Val
100         105         110

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atg gac ctg gtg gcc aac cac acc agc gac gag cac ccg tgg ttc atc	384
Met Asp Leu Val Ala Asn His Thr Ser Asp Glu His Pro Trp Phe Ile	
115 120 125	
gag agc cgc agc agc cgc gac aac ccg tac cgc gac tgg tac atc tgg	432
Glu Ser Arg Ser Ser Arg Asp Asn Pro Tyr Arg Asp Trp Tyr Ile Trp	
130 135 140	
cgc gac ccg aag gac ggc cgc gag ccg aac aac tgg ctg tcc tac ttc	480
Arg Asp Pro Lys Asp Gly Arg Glu Pro Asn Asn Trp Leu Ser Tyr Phe	
145 150 155 160	
agc ggc agc gcc tgg gag tac gac gag cgc acc ggc cag tac tac ctc	528
Ser Gly Ser Ala Trp Glu Tyr Asp Glu Arg Thr Gly Gln Tyr Tyr Leu	
165 170 175	
cac ctg ttc agc agg cgc cag ccg gac ctg aac tgg gag aac cct aaa	576
His Leu Phe Ser Arg Arg Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys	
180 185 190	
gtt cgc gag gcc atc ttc gag atg atg cgc ttc tgg ctg gac aag ggc	624
Val Arg Glu Ala Ile Phe Glu Met Met Arg Phe Trp Leu Asp Lys Gly	
195 200 205	
atc gac ggc ttc cgc atg gac gtg atc aac gcg atc gcc aag gcc gag	672
Ile Asp Gly Phe Arg Met Asp Val Ile Asn Ala Ile Ala Lys Ala Glu	
210 215 220	
ggc ctg cca gac gcc cca gct agg cct ggg gag aga tac gct tgg ggt	720
Gly Leu Pro Asp Ala Pro Ala Arg Pro Gly Glu Arg Tyr Ala Trp Gly	
225 230 235 240	
ggc cag tac ttc ctg aac cag ccg aag gtg cac gag tac ctc cgc gag	768
Gly Gln Tyr Phe Leu Asn Gln Pro Lys Val His Glu Tyr Leu Arg Glu	
245 250 255	
atg tac gac aag gtg ctg tcc cac tac gac atc atg act gtg ggg gag	816
Met Tyr Asp Lys Val Leu Ser His Tyr Asp Ile Met Thr Val Gly Glu	
260 265 270	
act ggg ggt gtc acc acc aag gac gcc ctg ctg ttc gct ggg gag gac	864
Thr Gly Gly Val Thr Thr Lys Asp Ala Leu Leu Phe Ala Gly Glu Asp	
275 280 285	
agg cgc gag ctg aac atg gtg ttc cag ttc gag cac atg gac atc gac	912
Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Ile Asp	
290 295 300	
gcc acc gac ggc gac aag tgg agg cca agg ccg tgg agg ctg acc gag	960
Ala Thr Asp Gly Asp Lys Trp Arg Pro Arg Pro Trp Arg Leu Thr Glu	
305 310 315 320	
ctt aag acc atc atg acc cgc tgg cag aac gac ctg tac ggc aag gcc	1008
Leu Lys Thr Ile Met Thr Arg Trp Gln Asn Asp Leu Tyr Gly Lys Ala	
325 330 335	
tgg aac agc ctg tac tgg acc aac cac gac cag ccg agg gcc gtg tcc	1056
Trp Asn Ser Leu Tyr Trp Thr Asn His Asp Gln Pro Arg Ala Val Ser	
340 345 350	
cgc ttc ggc aac gac ggc ccg tac cgc gtg gag agc gcc aag atg ctg	1104
Arg Phe Gly Asn Asp Gly Pro Tyr Arg Val Glu Ser Ala Lys Met Leu	
355 360 365	
gcc acc gtg ctg cac atg atg cag ggc acc ccg tac atc tac cag ggc	1152
Ala Thr Val Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly	
370 375 380	
gaa gaa atc ggc atg acc aat tgc cct ttc gac agc atc gac gag tac	1200
Glu Glu Ile Gly Met Thr Asn Cys Pro Phe Asp Ser Ile Asp Glu Tyr	
385 390 395 400	
cgc gac gtg gag atc cac aac ctt tgg aga cat cgc gtg atg gaa ggc	1248
Arg Asp Val Glu Ile His Asn Leu Trp Arg His Arg Val Met Glu Gly	
405 410 415	
ggc cag gac cct gct gag gtg ctg cgc gtg atc cag ctg aag ggc cgc	1296
Gly Gln Asp Pro Ala Glu Val Leu Arg Val Ile Gln Leu Lys Gly Arg	

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420	425	430	
gac aac gcc agg acc ccg atg cag tgg gac gac tcc ccg aac gcc ggc Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Pro Asn Ala Gly 435 440 445			1344
ttt acg acc ggg act cct tgg atc aag gtg aac ccg aac tac cgc gag Phe Thr Thr Gly Thr Pro Trp Ile Lys Val Asn Pro Asn Tyr Arg Glu 450 455 460			1392
atc aac gtg aag cag gct ctg gct gat ccg aac agc atc ttc cac tac Ile Asn Val Lys Gln Ala Leu Ala Asp Pro Asn Ser Ile Phe His Tyr 465 470 475 480			1440
tac cgc cgc ctc att cag ctc cgc aag cag cac ccg atc gtg gtg tac Tyr Arg Arg Leu Ile Gln Leu Arg Lys Gln His Pro Ile Val Val Tyr 485 490 495			1488
ggc aag tac gac ctg atc ctg ccg gac cac gag gaa atc tgg gcc tac Gly Lys Tyr Asp Leu Ile Leu Pro Asp His Glu Glu Ile Trp Ala Tyr 500 505 510			1536
acc cgc acc ctg ggc gac gag cgc tgg ctg atc gtg gcc aac ttc ttc Thr Arg Thr Leu Gly Asp Glu Arg Trp Leu Ile Val Ala Asn Phe Phe 515 520 525			1584
ggc ggc acc ccc gag ttc gag ctg ccg cca gaa gtg cgc tgc gag ggc Gly Gly Thr Pro Glu Phe Glu Leu Pro Pro Glu Val Arg Cys Glu Gly 530 535 540			1632
gca gaa ctt gtc att gcc aat tac ccg gtg gac gac agc gag gct ggc Ala Glu Leu Val Ile Ala Asn Tyr Pro Val Asp Asp Ser Glu Ala Gly 545 550 555 560			1680
ggc cca gct gct gct ggc gct ccg cac agg ttc agg ctg cgc ccc tac Gly Pro Ala Ala Ala Gly Ala Pro His Arg Phe Arg Leu Arg Pro Tyr 565 570 575			1728
gag tgc cgc gtg tac cgc ctg ctg ggc tgg cac Glu Cys Arg Val Tyr Arg Leu Leu Gly Trp His 580 585			1761

<210> SEQ ID NO 51

<211> LENGTH: 587

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 51

Met Ser Thr Ala Leu Thr Gln Thr Ser Thr Asn Ser Gln Gln Ser Pro
1 5 10 15

Ile Arg Arg Ala Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro
20 25 30

Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Ile Gly Asp Leu Arg Gly
35 40 45

Ile Leu Ser Lys Leu Asp Tyr Leu Lys Leu Leu Gly Val Asp Val Leu
50 55 60

Trp Leu Asn Pro Ile Tyr Asp Ser Pro Asn Asp Asp Met Gly Tyr Asp
65 70 75 80

Ile Arg Asp Tyr Tyr Lys Ile Met Glu Glu Phe Gly Thr Met Glu Asp
85 90 95

Phe Glu Glu Leu Leu Arg Glu Val His Ala Arg Gly Met Lys Leu Val
100 105 110

Met Asp Leu Val Ala Asn His Thr Ser Asp Glu His Pro Trp Phe Ile
115 120 125

Glu Ser Arg Ser Ser Arg Asp Asn Pro Tyr Arg Asp Trp Tyr Ile Trp
130 135 140

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Arg	Asp	Pro	Lys	Asp	Gly	Arg	Glu	Pro	Asn	Asn	Trp	Leu	Ser	Tyr	Phe	145	150	155	160
Ser	Gly	Ser	Ala	Trp	Glu	Tyr	Asp	Glu	Arg	Thr	Gly	Gln	Tyr	Tyr	Leu	165	170	175	
His	Leu	Phe	Ser	Arg	Arg	Gln	Pro	Asp	Leu	Asn	Trp	Glu	Asn	Pro	Lys	180	185	190	
Val	Arg	Glu	Ala	Ile	Phe	Glu	Met	Met	Arg	Phe	Trp	Leu	Asp	Lys	Gly	195	200	205	
Ile	Asp	Gly	Phe	Arg	Met	Asp	Val	Ile	Asn	Ala	Ile	Ala	Lys	Ala	Glu	210	215	220	
Gly	Leu	Pro	Asp	Ala	Pro	Ala	Arg	Pro	Gly	Glu	Arg	Tyr	Ala	Trp	Gly	225	230	235	240
Gly	Gln	Tyr	Phe	Leu	Asn	Gln	Pro	Lys	Val	His	Glu	Tyr	Leu	Arg	Glu	245	250	255	
Met	Tyr	Asp	Lys	Val	Leu	Ser	His	Tyr	Asp	Ile	Met	Thr	Val	Gly	Glu	260	265	270	
Thr	Gly	Gly	Val	Thr	Thr	Lys	Asp	Ala	Leu	Leu	Phe	Ala	Gly	Glu	Asp	275	280	285	
Arg	Arg	Glu	Leu	Asn	Met	Val	Phe	Gln	Phe	Glu	His	Met	Asp	Ile	Asp	290	295	300	
Ala	Thr	Asp	Gly	Asp	Lys	Trp	Arg	Pro	Arg	Pro	Trp	Arg	Leu	Thr	Glu	305	310	315	320
Leu	Lys	Thr	Ile	Met	Thr	Arg	Trp	Gln	Asn	Asp	Leu	Tyr	Gly	Lys	Ala	325	330	335	
Trp	Asn	Ser	Leu	Tyr	Trp	Thr	Asn	His	Asp	Gln	Pro	Arg	Ala	Val	Ser	340	345	350	
Arg	Phe	Gly	Asn	Asp	Gly	Pro	Tyr	Arg	Val	Glu	Ser	Ala	Lys	Met	Leu	355	360	365	
Ala	Thr	Val	Leu	His	Met	Met	Gln	Gly	Thr	Pro	Tyr	Ile	Tyr	Gln	Gly	370	375	380	
Glu	Glu	Ile	Gly	Met	Thr	Asn	Cys	Pro	Phe	Asp	Ser	Ile	Asp	Glu	Tyr	385	390	395	400
Arg	Asp	Val	Glu	Ile	His	Asn	Leu	Trp	Arg	His	Arg	Val	Met	Glu	Gly	405	410	415	
Gly	Gln	Asp	Pro	Ala	Glu	Val	Leu	Arg	Val	Ile	Gln	Leu	Lys	Gly	Arg	420	425	430	
Asp	Asn	Ala	Arg	Thr	Pro	Met	Gln	Trp	Asp	Asp	Ser	Pro	Asn	Ala	Gly	435	440	445	
Phe	Thr	Thr	Gly	Thr	Pro	Trp	Ile	Lys	Val	Asn	Pro	Asn	Tyr	Arg	Glu	450	455	460	
Ile	Asn	Val	Lys	Gln	Ala	Leu	Ala	Asp	Pro	Asn	Ser	Ile	Phe	His	Tyr	465	470	475	480
Tyr	Arg	Arg	Leu	Ile	Gln	Leu	Arg	Lys	Gln	His	Pro	Ile	Val	Val	Tyr	485	490	495	
Gly	Lys	Tyr	Asp	Leu	Ile	Leu	Pro	Asp	His	Glu	Glu	Ile	Trp	Ala	Tyr	500	505	510	
Thr	Arg	Thr	Leu	Gly	Asp	Glu	Arg	Trp	Leu	Ile	Val	Ala	Asn	Phe	Phe	515	520	525	
Gly	Gly	Thr	Pro	Glu	Phe	Glu	Leu	Pro	Pro	Glu	Val	Arg	Cys	Glu	Gly	530	535	540	
Ala	Glu	Leu	Val	Ile	Ala	Asn	Tyr	Pro	Val	Asp	Asp	Ser	Glu	Ala	Gly	545	550	555	560
Gly	Pro	Ala	Ala	Ala	Gly	Ala	Pro	His	Arg	Phe	Arg	Leu	Arg	Pro	Tyr				

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565	570	575	
Glu Cys Arg Val Tyr Arg Leu Leu Gly Trp His			
580	585		
 <210> SEQ ID NO 52			
<211> LENGTH: 6			
<212> TYPE: PRT			
<213> ORGANISM: unknown			
<220> FEATURE:			
<223> OTHER INFORMATION: synthetic sequence			
<220> FEATURE:			
<221> NAME/KEY: signal			
<222> LOCATION: (1)..(6)			
<223> OTHER INFORMATION: ER retention sequence			
 <400> SEQUENCE: 52			
Ser Glu Lys Asp Glu Leu			
1	5		
 <210> SEQ ID NO 53			
<211> LENGTH: 1764			
<212> TYPE: DNA			
<213> ORGANISM: unknown			
<220> FEATURE:			
<223> OTHER INFORMATION: synthetic gene			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(1764)			
<223> OTHER INFORMATION: dicot optimized alpha-1,1-glucosidase BSAM1606			
 <400> SEQUENCE: 53			
atg tct act gct ctt act cag act tct act aac tct cag cag tct cca			48
Met Ser Thr Ala Leu Thr Gln Thr Ser Thr Asn Ser Gln Gln Ser Pro			
1	5	10	15
att aga agg gct tgg tgg aaa gag gct gtt gtt tac caa atc tac cca			96
Ile Arg Arg Ala Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro			
	20	25	30
cgt tct ttc atg gat tcc aac ggt gat gga att gga gat ctt agg gga			144
Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Ile Gly Asp Leu Arg Gly			
	35	40	45
att ctc tcc aag ttg gat tac ctt aag ttg ctc gga gtt gat gtt ctt			192
Ile Leu Ser Lys Leu Asp Tyr Leu Lys Leu Leu Gly Val Asp Val Leu			
	50	55	60
tgg ctc aac cca atc tac gat tcc cca aac gat gat atg gga tac gat			240
Trp Leu Asn Pro Ile Tyr Asp Ser Pro Asn Asp Asp Met Gly Tyr Asp			
65	70	75	80
atc agg gat tac tac aag atc atg gaa gag ttc gga act atg gaa gat			288
Ile Arg Asp Tyr Tyr Lys Ile Met Glu Glu Phe Gly Thr Met Glu Asp			
	85	90	95
ttc gag gaa ctt ctt aga gaa gtt cac gct cgt gga atg aag ttg gtg			336
Phe Glu Glu Leu Leu Arg Glu Val His Ala Arg Gly Met Lys Leu Val			
	100	105	110
atg gat ctt gtt gct aac cac act tct gat gag cac cct tgg ttt att			384
Met Asp Leu Val Ala Asn His Thr Ser Asp Glu His Pro Trp Phe Ile			
	115	120	125
gag tct agg tcc tct agg gat aat cca tac cgt gat tgg tac att tgg			432
Glu Ser Arg Ser Ser Arg Asp Asn Pro Tyr Arg Asp Trp Tyr Ile Trp			
	130	135	140
cgt gat cca aag gat gga aga gag cca aat aac tgg ctt tct tac ttc			480
Arg Asp Pro Lys Asp Gly Arg Glu Pro Asn Asn Trp Leu Ser Tyr Phe			
145	150	155	160
tct gga tct gct tgg gaa tat gat gag agg act gga cag tac tac ctt			528
Ser Gly Ser Ala Trp Glu Tyr Asp Glu Arg Thr Gly Gln Tyr Tyr Leu			
	165	170	175

-continued

cac ttg ttc tct aga agg cag cca gat ctt aat tgg gag aac cca aaa	576
His Leu Phe Ser Arg Arg Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys	
180 185 190	
gtg cgt gaa gct atc ttt gag atg atg agg ttc tgg ctc gat aag gga	624
Val Arg Glu Ala Ile Phe Glu Met Met Arg Phe Trp Leu Asp Lys Gly	
195 200 205	
att gat gga ttc agg atg gat gtg atc aac gct att gct aag gct gaa	672
Ile Asp Gly Phe Arg Met Asp Val Ile Asn Ala Ile Ala Lys Ala Glu	
210 215 220	
gga ctt cca gat gct cca gct aga cca ggt gaa aga tat gct tgg gga	720
Gly Leu Pro Asp Ala Pro Ala Arg Pro Gly Glu Arg Tyr Ala Trp Gly	
225 230 235 240	
gga cag tat ttc ctt aac cag cca aag gtt cac gaa tac ctc aga gag	768
Gly Gln Tyr Phe Leu Asn Gln Pro Lys Val His Glu Tyr Leu Arg Glu	
245 250 255	
atg tac gat aag gtt ctc tcc cac tac gat att atg act gtg gga gag	816
Met Tyr Asp Lys Val Leu Ser His Tyr Asp Ile Met Thr Val Gly Glu	
260 265 270	
act ggt gga gtt act act aag gat gct ctc ttg ttc gca ggc gaa gat	864
Thr Gly Gly Val Thr Thr Lys Asp Ala Leu Leu Phe Ala Gly Glu Asp	
275 280 285	
aga agg gaa ctc aac atg gtt ttc cag ttc gag cac atg gat atc gat	912
Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Ile Asp	
290 295 300	
gct act gat ggt gat aag tgg agg cca aga cct tgg aga ctt act gag	960
Ala Thr Asp Gly Asp Lys Trp Arg Pro Arg Pro Trp Arg Leu Thr Glu	
305 310 315 320	
ctt aag act atc atg act agg tgg cag aat gat ctt tat gga aag gct	1008
Leu Lys Thr Ile Met Thr Arg Trp Gln Asn Asp Leu Tyr Gly Lys Ala	
325 330 335	
tgg aac tct ctc tac tgg act aat cat gat cag cca agg gct gtt tct	1056
Trp Asn Ser Leu Tyr Trp Thr Asn His Asp Gln Pro Arg Ala Val Ser	
340 345 350	
aga ttc gga aac gat gga cca tat cgt gtt gag tct gct aag atg ctt	1104
Arg Phe Gly Asn Asp Gly Pro Tyr Arg Val Glu Ser Ala Lys Met Leu	
355 360 365	
gct act gtg ctt cat atg atg caa ggt aca cct tac atc tac cag ggt	1152
Ala Thr Val Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly	
370 375 380	
gaa gag att gga atg act aac tgc cca ttc gat tcc att gat gag tac	1200
Glu Glu Ile Gly Met Thr Asn Cys Pro Phe Asp Ser Ile Asp Glu Tyr	
385 390 395 400	
cgt gat gtg gag att cat aac ctt tgg agg cac aga gtt atg gaa ggt	1248
Arg Asp Val Glu Ile His Asn Leu Trp Arg His Arg Val Met Glu Gly	
405 410 415	
gga caa gat cca gct gaa gtt ctt agg gtg atc caa ctt aag gga agg	1296
Gly Gln Asp Pro Ala Glu Val Leu Arg Val Ile Gln Leu Lys Gly Arg	
420 425 430	
gat aat gct aga act cca atg caa tgg gat gat tct cca aac gct gga	1344
Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Pro Asn Ala Gly	
435 440 445	
ttc act act gga aca cct tgg att aag gtg aac cca aac tac aga gag	1392
Phe Thr Thr Gly Thr Pro Trp Ile Lys Val Asn Pro Asn Tyr Arg Glu	
450 455 460	
atc aac gtt aag cag gct ctt gct gat cca aac tcc atc ttc cat tac	1440
Ile Asn Val Lys Gln Ala Leu Ala Asp Pro Asn Ser Ile Phe His Tyr	
465 470 475 480	
tac cgt aga ctt atc caa ctt agg aag cag cat cca atc gtt gtt tac	1488
Tyr Arg Arg Leu Ile Gln Leu Arg Lys Gln His Pro Ile Val Val Tyr	

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485	490	495	
gga aag tac gat ctc att ctc cca gat cac gaa gag att tgg gct tac Gly Lys Tyr Asp Leu Ile Leu Pro Asp His Glu Glu Ile Trp Ala Tyr 500 505 510			1536
act agg act ctt gga gat gag aga tgg ctt atc gtg gct aat ttc ttc Thr Arg Thr Leu Gly Asp Glu Arg Trp Leu Ile Val Ala Asn Phe Phe 515 520 525			1584
gga gga act cca gaa ttt gaa ctt cca cct gaa gtt aga tgt gag ggt Gly Gly Thr Pro Glu Phe Glu Leu Pro Pro Glu Val Arg Cys Glu Gly 530 535 540			1632
gct gag ttg gtt att gct aac tac cca gtg gat gat tct gaa gct ggc Ala Glu Leu Val Ile Ala Asn Tyr Pro Val Asp Asp Ser Glu Ala Gly 545 550 555 560			1680
ggt cct gct gct gct ggt gct cca cat agg ttt agg ctt agg cca tat Gly Pro Ala Ala Ala Gly Ala Pro His Arg Phe Arg Leu Arg Pro Tyr 565 570 575			1728
gag tgt cgt gtt tac cgt ctt ttg gga tgg cat taa Glu Cys Arg Val Tyr Arg Leu Leu Gly Trp His 580 585			1764

<210> SEQ ID NO 54

<211> LENGTH: 587

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 54

Met Ser Thr Ala Leu Thr Gln Thr Ser Thr Asn Ser Gln Gln Ser Pro 1 5 10 15	
Ile Arg Arg Ala Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro 20 25 30	
Arg Ser Phe Met Asp Ser Asn Gly Asp Gly Ile Gly Asp Leu Arg Gly 35 40 45	
Ile Leu Ser Lys Leu Asp Tyr Leu Lys Leu Leu Gly Val Asp Val Leu 50 55 60	
Trp Leu Asn Pro Ile Tyr Asp Ser Pro Asn Asp Asp Met Gly Tyr Asp 65 70 75 80	
Ile Arg Asp Tyr Tyr Lys Ile Met Glu Glu Phe Gly Thr Met Glu Asp 85 90 95	
Phe Glu Glu Leu Leu Arg Glu Val His Ala Arg Gly Met Lys Leu Val 100 105 110	
Met Asp Leu Val Ala Asn His Thr Ser Asp Glu His Pro Trp Phe Ile 115 120 125	
Glu Ser Arg Ser Ser Arg Asp Asn Pro Tyr Arg Asp Trp Tyr Ile Trp 130 135 140	
Arg Asp Pro Lys Asp Gly Arg Glu Pro Asn Asn Trp Leu Ser Tyr Phe 145 150 155 160	
Ser Gly Ser Ala Trp Glu Tyr Asp Glu Arg Thr Gly Gln Tyr Tyr Leu 165 170 175	
His Leu Phe Ser Arg Arg Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys 180 185 190	
Val Arg Glu Ala Ile Phe Glu Met Met Arg Phe Trp Leu Asp Lys Gly 195 200 205	
Ile Asp Gly Phe Arg Met Asp Val Ile Asn Ala Ile Ala Lys Ala Glu 210 215 220	
Gly Leu Pro Asp Ala Pro Ala Arg Pro Gly Glu Arg Tyr Ala Trp Gly	

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225	230	235	240
Gly Gln Tyr Phe Leu Asn Gln Pro Lys Val His Glu Tyr Leu Arg Glu	245	250	255
Met Tyr Asp Lys Val Leu Ser His Tyr Asp Ile Met Thr Val Gly Glu	260	265	270
Thr Gly Gly Val Thr Thr Lys Asp Ala Leu Leu Phe Ala Gly Glu Asp	275	280	285
Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Ile Asp	290	295	300
Ala Thr Asp Gly Asp Lys Trp Arg Pro Arg Pro Trp Arg Leu Thr Glu	305	310	315
Leu Lys Thr Ile Met Thr Arg Trp Gln Asn Asp Leu Tyr Gly Lys Ala	325	330	335
Trp Asn Ser Leu Tyr Trp Thr Asn His Asp Gln Pro Arg Ala Val Ser	340	345	350
Arg Phe Gly Asn Asp Gly Pro Tyr Arg Val Glu Ser Ala Lys Met Leu	355	360	365
Ala Thr Val Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly	370	375	380
Glu Glu Ile Gly Met Thr Asn Cys Pro Phe Asp Ser Ile Asp Glu Tyr	385	390	395
Arg Asp Val Glu Ile His Asn Leu Trp Arg His Arg Val Met Glu Gly	405	410	415
Gly Gln Asp Pro Ala Glu Val Leu Arg Val Ile Gln Leu Lys Gly Arg	420	425	430
Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Pro Asn Ala Gly	435	440	445
Phe Thr Thr Gly Thr Pro Trp Ile Lys Val Asn Pro Asn Tyr Arg Glu	450	455	460
Ile Asn Val Lys Gln Ala Leu Ala Asp Pro Asn Ser Ile Phe His Tyr	465	470	475
Tyr Arg Arg Leu Ile Gln Leu Arg Lys Gln His Pro Ile Val Val Tyr	485	490	495
Gly Lys Tyr Asp Leu Ile Leu Pro Asp His Glu Glu Ile Trp Ala Tyr	500	505	510
Thr Arg Thr Leu Gly Asp Glu Arg Trp Leu Ile Val Ala Asn Phe Phe	515	520	525
Gly Gly Thr Pro Glu Phe Glu Leu Pro Pro Glu Val Arg Cys Glu Gly	530	535	540
Ala Glu Leu Val Ile Ala Asn Tyr Pro Val Asp Asp Ser Glu Ala Gly	545	550	555
Gly Pro Ala Ala Ala Gly Ala Pro His Arg Phe Arg Leu Arg Pro Tyr	565	570	575
Glu Cys Arg Val Tyr Arg Leu Leu Gly Trp His	580	585	

<210> SEQ ID NO 55

<211> LENGTH: 1764

<212> TYPE: DNA

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: synthetic gene

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1764)

<223> OTHER INFORMATION: monocot optimized alpha-1,6-glucosidase

-continued

<400> SEQUENCE: 55

atg ggc cgc gtg ctg ctc gtg gcc ctg gcc ctg ctc gct ctc gcc gcc	48
Met Gly Arg Val Leu Leu Val Ala Leu Ala Leu Leu Ala Leu Ala Ala	
1 5 10 15	
agc gct acc tct gaa aga gtg tgg tgg aag gaa gcc gtc gtc tac cag	96
Ser Ala Thr Ser Glu Arg Val Trp Trp Lys Glu Ala Val Val Tyr Gln	
20 25 30	
atc tac ccg cgc agc ttc tac gac agc aac gcc gac gcc atc gcc gac	144
Ile Tyr Pro Arg Ser Phe Tyr Asp Ser Asn Gly Asp Gly Ile Gly Asp	
35 40 45	
atc cgc gcc atc att gcc aag ctg gac tac ctg aag gaa ctg gcc gtc	192
Ile Arg Gly Ile Ile Ala Lys Leu Asp Tyr Leu Lys Glu Leu Gly Val	
50 55 60	
gac gtt gtg tgg ctg tcc ccg gtg tac aag agc ccg aac gat gac aat	240
Asp Val Val Trp Leu Ser Pro Val Tyr Lys Ser Pro Asn Asp Asp Asn	
65 70 75 80	
ggc tac gat atc tcc gac tac cgc gac atc atg gac gag ttc gcc acg	288
Gly Tyr Asp Ile Ser Asp Tyr Arg Asp Ile Met Asp Glu Phe Gly Thr	
85 90 95	
atg gcc gac tgg aag acc atg ctc gag gaa atg cac aag cgc gcc atc	336
Met Ala Asp Trp Lys Thr Met Leu Glu Glu Met His Lys Arg Gly Ile	
100 105 110	
aag ctg gtg atg gac ctg gtg gtg aac cac acc agc gac gag cac ccg	384
Lys Leu Val Met Asp Leu Val Val Asn His Thr Ser Asp Glu His Pro	
115 120 125	
tgg ttc atc gag agc cgc aag agc aag gac aac ccg tac cgc gac tac	432
Trp Phe Ile Glu Ser Arg Lys Ser Lys Asp Asn Pro Tyr Arg Asp Tyr	
130 135 140	
tac atc tgg cgc cca gcc aag aac gcc aag gaa ccg aac aac tgg gag	480
Tyr Ile Trp Arg Pro Gly Lys Asn Gly Lys Glu Pro Asn Asn Trp Glu	
145 150 155 160	
agc gtg ttc agc gcc agc gcc tgg gag tac gac gag atg acc gcc gag	528
Ser Val Phe Ser Gly Ser Ala Trp Glu Tyr Asp Glu Met Thr Gly Glu	
165 170 175	
tac tac ctc cac ctg ttc agc aag aag cag ccg gac ctg aac tgg gag	576
Tyr Tyr Leu His Leu Phe Ser Lys Lys Gln Pro Asp Leu Asn Trp Glu	
180 185 190	
aac ccg aag gtg cgc cgc gag gtg tac gag atg atg aag ttc tgg ctg	624
Asn Pro Lys Val Arg Arg Glu Val Tyr Glu Met Met Lys Phe Trp Leu	
195 200 205	
gac aag gcc gtg gac gcc ttc cgc atg gac gtg atc aac atg atc agc	672
Asp Lys Gly Val Asp Gly Phe Arg Met Asp Val Ile Asn Met Ile Ser	
210 215 220	
aag gtg ccc gag ctg cca gat gcc gag ccg cag agc gcc aag aag tac	720
Lys Val Pro Glu Leu Pro Asp Gly Glu Pro Gln Ser Gly Lys Lys Tyr	
225 230 235 240	
gcc tct gcc tcc cgc tac tac atg aac gcc ccg agg gtg cac gag ttc	768
Ala Ser Gly Ser Arg Tyr Tyr Met Asn Gly Pro Arg Val His Glu Phe	
245 250 255	
ctc caa gaa atg aat cgc gaa gtg ctc tcc aag tac gac atc atg act	816
Leu Gln Glu Met Asn Arg Glu Val Leu Ser Lys Tyr Asp Ile Met Thr	
260 265 270	
gtg gcc gag act ccg gcc gtg acc ccg aag gaa gcc atc ctg tac acc	864
Val Gly Glu Thr Pro Gly Val Thr Pro Lys Glu Gly Ile Leu Tyr Thr	
275 280 285	
gac ccg agc agg cgc gag ctg aac atg gtg ttc cag ttc gag cac atg	912
Asp Pro Ser Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met	
290 295 300	

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gac ctc gac tct ggt cca ggc ggc aag tgg gac atc agg ccg tgg agc	960
Asp Leu Asp Ser Gly Pro Gly Gly Lys Trp Asp Ile Arg Pro Trp Ser	
305 310 315 320	
ctg gcc gac ctg aag aag acc atg acc aag tgg cag aag gaa ctt gag	1008
Leu Ala Asp Leu Lys Lys Thr Met Thr Lys Trp Gln Lys Glu Leu Glu	
325 330 335	
ggc aag ggc tgg aac agc ctg tac ctg aac aac cac gac cag ccg agg	1056
Gly Lys Gly Trp Asn Ser Leu Tyr Leu Asn Asn His Asp Gln Pro Arg	
340 345 350	
gcc gtg tcc aga ttc ggc gac gac ggc aag tac cgc gtg gag agc gcc	1104
Ala Val Ser Arg Phe Gly Asp Asp Gly Lys Tyr Arg Val Glu Ser Ala	
355 360 365	
aag atg ctg gcc acc ttc ctg cac atg atg caa ggc acc ccg tac atc	1152
Lys Met Leu Ala Thr Phe Leu His Met Met Gln Gly Thr Pro Tyr Ile	
370 375 380	
tac cag ggc gaa gaa atc ggc atg acc aat gtg cgc ttc ccg agc atc	1200
Tyr Gln Gly Glu Glu Ile Gly Met Thr Asn Val Arg Phe Pro Ser Ile	
385 390 395 400	
gag gac tac cgg gac atc gag act ctg aac atg tac aag gaa cgc gtc	1248
Glu Asp Tyr Arg Asp Ile Glu Thr Leu Asn Met Tyr Lys Glu Arg Val	
405 410 415	
gag gaa tac ggc gag gac ccg caa gag gtg atg gaa aag atc tac tac	1296
Glu Glu Tyr Gly Glu Asp Pro Gln Glu Val Met Glu Lys Ile Tyr Tyr	
420 425 430	
aag ggc cgc gac aac gcc agg acc ccg atg caa tgg gac gac agc gag	1344
Lys Gly Arg Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Glu	
435 440 445	
aac gcc ggc ttc acc gcc ggc acc ccg tgg att cgc gtg aac ccg aac	1392
Asn Ala Gly Phe Thr Ala Gly Thr Pro Trp Ile Pro Val Asn Pro Asn	
450 455 460	
tac aag gaa atc aac gtc aag gcc gcc ctc gaa gat cca aac agc gtg	1440
Tyr Lys Glu Ile Asn Val Lys Ala Ala Leu Glu Asp Pro Asn Ser Val	
465 470 475 480	
ttc cac tac tac aag aag ctg atc cag ctg cgc aag cag cac gac atc	1488
Phe His Tyr Tyr Lys Lys Leu Ile Gln Leu Arg Lys Gln His Asp Ile	
485 490 495	
atc gtg tac ggc acc tac gac ctg atc ctc gag gac gac cct tac atc	1536
Ile Val Tyr Gly Thr Tyr Asp Leu Ile Leu Glu Asp Asp Pro Tyr Ile	
500 505 510	
tac cgc tac acc cgc acc ctg ggc aac gag cag ctg atc gtg atc acc	1584
Tyr Arg Tyr Thr Arg Thr Leu Gly Asn Glu Gln Leu Ile Val Ile Thr	
515 520 525	
aac ttc agc gaa aag acc ccg gtg ttc cgc ctg ccg gac cac atc atc	1632
Asn Phe Ser Glu Lys Thr Pro Val Phe Arg Leu Pro Asp His Ile Ile	
530 535 540	
tac aag acc aag gaa ctc ctc atc tct aac tac gac gtg gac gag gcc	1680
Tyr Lys Thr Lys Glu Leu Leu Ile Ser Asn Tyr Asp Val Asp Glu Ala	
545 550 555 560	
gag gaa ctg aag gaa atc agg ctg agg ccc tgg gag gcc cgc gtg tac	1728
Glu Glu Leu Lys Glu Ile Arg Leu Arg Pro Trp Glu Ala Arg Val Tyr	
565 570 575	
aag atc agg ctg cca agc gag aag gac gag ctg tga	1764
Lys Ile Arg Leu Pro Ser Glu Lys Asp Glu Leu	
580 585	

<210> SEQ ID NO 56

<211> LENGTH: 587

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

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<400> SEQUENCE: 56

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Met Gly Arg Val Leu Leu Val Ala Leu Ala Leu Leu Ala Leu Ala Ala
1      5      10      15

Ser Ala Thr Ser Glu Arg Val Trp Trp Lys Glu Ala Val Val Tyr Gln
20      25      30

Ile Tyr Pro Arg Ser Phe Tyr Asp Ser Asn Gly Asp Gly Ile Gly Asp
35      40      45

Ile Arg Gly Ile Ile Ala Lys Leu Asp Tyr Leu Lys Glu Leu Gly Val
50      55      60

Asp Val Val Trp Leu Ser Pro Val Tyr Lys Ser Pro Asn Asp Asp Asn
65      70      75      80

Gly Tyr Asp Ile Ser Asp Tyr Arg Asp Ile Met Asp Glu Phe Gly Thr
85      90      95

Met Ala Asp Trp Lys Thr Met Leu Glu Glu Met His Lys Arg Gly Ile
100     105     110

Lys Leu Val Met Asp Leu Val Val Asn His Thr Ser Asp Glu His Pro
115     120     125

Trp Phe Ile Glu Ser Arg Lys Ser Lys Asp Asn Pro Tyr Arg Asp Tyr
130     135     140

Tyr Ile Trp Arg Pro Gly Lys Asn Gly Lys Glu Pro Asn Asn Trp Glu
145     150     155     160

Ser Val Phe Ser Gly Ser Ala Trp Glu Tyr Asp Glu Met Thr Gly Glu
165     170     175

Tyr Tyr Leu His Leu Phe Ser Lys Lys Gln Pro Asp Leu Asn Trp Glu
180     185     190

Asn Pro Lys Val Arg Arg Glu Val Tyr Glu Met Met Lys Phe Trp Leu
195     200     205

Asp Lys Gly Val Asp Gly Phe Arg Met Asp Val Ile Asn Met Ile Ser
210     215     220

Lys Val Pro Glu Leu Pro Asp Gly Glu Pro Gln Ser Gly Lys Lys Tyr
225     230     235     240

Ala Ser Gly Ser Arg Tyr Tyr Met Asn Gly Pro Arg Val His Glu Phe
245     250     255

Leu Gln Glu Met Asn Arg Glu Val Leu Ser Lys Tyr Asp Ile Met Thr
260     265     270

Val Gly Glu Thr Pro Gly Val Thr Pro Lys Glu Gly Ile Leu Tyr Thr
275     280     285

Asp Pro Ser Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met
290     295     300

Asp Leu Asp Ser Gly Pro Gly Gly Lys Trp Asp Ile Arg Pro Trp Ser
305     310     315     320

Leu Ala Asp Leu Lys Lys Thr Met Thr Lys Trp Gln Lys Glu Leu Glu
325     330     335

Gly Lys Gly Trp Asn Ser Leu Tyr Leu Asn Asn His Asp Gln Pro Arg
340     345     350

Ala Val Ser Arg Phe Gly Asp Asp Gly Lys Tyr Arg Val Glu Ser Ala
355     360     365

Lys Met Leu Ala Thr Phe Leu His Met Met Gln Gly Thr Pro Tyr Ile
370     375     380

Tyr Gln Gly Glu Glu Ile Gly Met Thr Asn Val Arg Phe Pro Ser Ile
385     390     395     400

Glu Asp Tyr Arg Asp Ile Glu Thr Leu Asn Met Tyr Lys Glu Arg Val

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405					410					415					
Glu	Glu	Tyr	Gly	Glu	Asp	Pro	Gln	Glu	Val	Met	Glu	Lys	Ile	Tyr	Tyr
			420					425					430		
Lys	Gly	Arg	Asp	Asn	Ala	Arg	Thr	Pro	Met	Gln	Trp	Asp	Asp	Ser	Glu
		435					440					445			
Asn	Ala	Gly	Phe	Thr	Ala	Gly	Thr	Pro	Trp	Ile	Pro	Val	Asn	Pro	Asn
		450				455					460				
Tyr	Lys	Glu	Ile	Asn	Val	Lys	Ala	Ala	Leu	Glu	Asp	Pro	Asn	Ser	Val
465				470						475					480
Phe	His	Tyr	Tyr	Lys	Lys	Leu	Ile	Gln	Leu	Arg	Lys	Gln	His	Asp	Ile
				485					490					495	
Ile	Val	Tyr	Gly	Thr	Tyr	Asp	Leu	Ile	Leu	Glu	Asp	Asp	Pro	Tyr	Ile
			500				505						510		
Tyr	Arg	Tyr	Thr	Arg	Thr	Leu	Gly	Asn	Glu	Gln	Leu	Ile	Val	Ile	Thr
		515					520					525			
Asn	Phe	Ser	Glu	Lys	Thr	Pro	Val	Phe	Arg	Leu	Pro	Asp	His	Ile	Ile
		530				535					540				
Tyr	Lys	Thr	Lys	Glu	Leu	Leu	Ile	Ser	Asn	Tyr	Asp	Val	Asp	Glu	Ala
545					550					555					560
Glu	Glu	Leu	Lys	Glu	Ile	Arg	Leu	Arg	Pro	Trp	Glu	Ala	Arg	Val	Tyr
			565						570					575	
Lys	Ile	Arg	Leu	Pro	Ser	Glu	Lys	Asp	Glu	Leu					
			580					585							

<210> SEQ ID NO 57

<211> LENGTH: 1689

<212> TYPE: DNA

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: synthetic gene

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1689)

<223> OTHER INFORMATION: monocot optimized alpha-1,6-glucosidase Geo

<400> SEQUENCE: 57

atg	gaa	aga	gtg	tgg	tgg	aag	gaa	gcc	gtc	gtc	tac	cag	atc	tac	ccg	48
Met	Glu	Arg	Val	Trp	Trp	Lys	Glu	Ala	Val	Val	Tyr	Gln	Ile	Tyr	Pro	
1			5						10				15			
cgc	agc	ttc	tac	gac	agc	aac	ggc	gac	ggc	atc	ggc	gac	atc	cgc	ggc	96
Arg	Ser	Phe	Tyr	Asp	Ser	Asn	Gly	Asp	Gly	Ile	Gly	Asp	Ile	Arg	Gly	
		20					25					30				
atc	att	gcc	aag	ctg	gac	tac	ctg	aag	gaa	ctg	ggc	gtc	gac	gtt	gtg	144
Ile	Ile	Ala	Lys	Leu	Asp	Tyr	Leu	Lys	Glu	Leu	Gly	Val	Asp	Val	Val	
		35					40					45				
tgg	ctg	tcc	ccg	gtg	tac	aag	agc	ccg	aac	gat	gac	aat	ggc	tac	gat	192
Trp	Leu	Ser	Pro	Val	Tyr	Lys	Ser	Pro	Asn	Asp	Asp	Asn	Gly	Tyr	Asp	
	50					55				60						
atc	tcc	gac	tac	cgc	gac	atc	atg	gac	gag	ttc	ggc	acg	atg	gcc	gac	240
Ile	Ser	Asp	Tyr	Arg	Asp	Ile	Met	Asp	Glu	Phe	Gly	Thr	Met	Ala	Asp	
65					70					75				80		
tgg	aag	acc	atg	ctc	gag	gaa	atg	cac	aag	cgc	ggc	atc	aag	ctg	gtg	288
Trp	Lys	Thr	Met	Leu	Glu	Glu	Met	His	Lys	Arg	Gly	Ile	Lys	Leu	Val	
			85					90					95			
atg	gac	ctg	gtg	gtg	aac	cac	acc	agc	gac	gag	cac	ccg	tgg	ttc	atc	336
Met	Asp	Leu	Val	Val	Asn	His	Thr	Ser	Asp	Glu	His	Pro	Trp	Phe	Ile	
		100						105					110			
gag	agc	cgc	aag	agc	aag	gac	aac	ccg	tac	cgc	gac	tac	tac	atc	tgg	384
Glu	Ser	Arg	Lys	Ser	Lys	Asp	Asn	Pro	Tyr	Arg	Asp	Tyr	Tyr	Ile	Trp	

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115	120	125	
cgc cca ggc aag aac ggc aag gaa ccg aac aac tgg gag agc gtg ttc Arg Pro Gly Lys Asn Gly Lys Glu Pro Asn Asn Trp Glu Ser Val Phe 130 135 140			432
agc ggc agc gcc tgg gag tac gac gag atg acc ggc gag tac tac ctc Ser Gly Ser Ala Trp Glu Tyr Asp Glu Met Thr Gly Glu Tyr Tyr Leu 145 150 155 160			480
cac ctg ttc agc aag aag cag ccg gac ctg aac tgg gag aac ccg aag His Leu Phe Ser Lys Lys Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys 165 170 175			528
gtg cgc cgc gag gtg tac gag atg atg aag ttc tgg ctg gac aag ggc Val Arg Arg Glu Val Tyr Glu Met Met Lys Phe Trp Leu Asp Lys Gly 180 185 190			576
gtg gac ggc ttc cgc atg gac gtg atc aac atg atc agc aag gtg ccc Val Asp Gly Phe Arg Met Asp Val Ile Asn Met Ile Ser Lys Val Pro 195 200 205			624
gag ctg cca gat ggc gag ccg cag agc ggc aag aag tac gcc tct ggc Glu Leu Pro Asp Gly Glu Pro Gln Ser Gly Lys Lys Tyr Ala Ser Gly 210 215 220			672
tcc cgc tac tac atg aac ggc ccg agg gtg cac gag ttc ctc caa gaa Ser Arg Tyr Tyr Met Asn Gly Pro Arg Val His Glu Phe Leu Gln Glu 225 230 235 240			720
atg aat cgc gaa gtg ctc tcc aag tac gac atc atg act gtg ggc gag Met Asn Arg Glu Val Leu Ser Lys Tyr Asp Ile Met Thr Val Gly Glu 245 250 255			768
act ccg ggc gtg acc ccg aag gaa ggc atc ctg tac acc gac ccg agc Thr Pro Gly Val Thr Pro Lys Glu Gly Ile Leu Tyr Thr Asp Pro Ser 260 265 270			816
agg cgc gag ctg aac atg gtg ttc cag ttc gag cac atg gac ctc gac Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Leu Asp 275 280 285			864
tct ggt cca ggc ggc aag tgg gac atc agg ccg tgg agc ctg gcc gac Ser Gly Pro Gly Gly Lys Trp Asp Ile Arg Pro Trp Ser Leu Ala Asp 290 295 300			912
ctg aag aag acc atg acc aag tgg cag aag gaa ctt gag ggc aag ggc Leu Lys Lys Thr Met Thr Lys Trp Gln Lys Glu Leu Glu Gly Lys Gly 305 310 315 320			960
tgg aac agc ctg tac ctg aac aac cac gac cag ccg agg gcc gtg tcc Trp Asn Ser Leu Tyr Leu Asn Asn His Asp Gln Pro Arg Ala Val Ser 325 330 335			1008
aga ttc ggc gac gac ggc aag tac cgc gtg gag agc gcc aag atg ctg Arg Phe Gly Asp Asp Gly Lys Tyr Arg Val Glu Ser Ala Lys Met Leu 340 345 350			1056
gcc acc ttc ctg cac atg atg caa ggc acc ccg tac atc tac cag ggc Ala Thr Phe Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly 355 360 365			1104
gaa gaa atc ggc atg acc aat gtg cgc ttc ccg agc atc gag gac tac Glu Glu Ile Gly Met Thr Asn Val Arg Phe Pro Ser Ile Glu Asp Tyr 370 375 380			1152
cgg gac atc gag act ctg aac atg tac aag gaa cgc gtc gag gaa tac Arg Asp Ile Glu Thr Leu Asn Met Tyr Lys Glu Arg Val Glu Glu Tyr 385 390 395 400			1200
ggc gag gac ccg caa gag gtg atg gaa aag atc tac tac aag ggc cgc Gly Glu Asp Pro Gln Glu Val Met Glu Lys Ile Tyr Tyr Lys Gly Arg 405 410 415			1248
gac aac gcc agg acc ccg atg caa tgg gac gac agc gag aac gcc ggc Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Glu Asn Ala Gly 420 425 430			1296
ttc acc gcc ggc acc ccg tgg att ccg gtg aac ccg aac tac aag gaa			1344

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Phe Thr Ala Gly Thr Pro Trp Ile Pro Val Asn Pro Asn Tyr Lys Glu	
435 440 445	
atc aac gtc aag gcc gcc ctc gaa gat cca aac agc gtg ttc cac tac	1392
Ile Asn Val Lys Ala Ala Leu Glu Asp Pro Asn Ser Val Phe His Tyr	
450 455 460	
tac aag aag ctg atc cag ctg cgc aag cag cac gac atc atc gtg tac	1440
Tyr Lys Lys Leu Ile Gln Leu Arg Lys Gln His Asp Ile Ile Val Tyr	
465 470 475 480	
ggc acc tac gac ctg atc ctc gag gac gac cct tac atc tac cgc tac	1488
Gly Thr Tyr Asp Leu Ile Leu Glu Asp Asp Pro Tyr Ile Tyr Arg Tyr	
485 490 495	
acc cgc acc ctg ggc aac gag cag ctg atc gtg atc acc aac ttc agc	1536
Thr Arg Thr Leu Gly Asn Glu Gln Leu Ile Val Ile Thr Asn Phe Ser	
500 505 510	
gaa aag acc ccg gtg ttc cgc ctg ccg gac cac atc atc tac aag acc	1584
Glu Lys Thr Pro Val Phe Arg Leu Pro Asp His Ile Ile Tyr Lys Thr	
515 520 525	
aag gaa ctc ctc atc tct aac tac gac gtg gac gag gcc gag gaa ctg	1632
Lys Glu Leu Leu Ile Ser Asn Tyr Asp Val Asp Glu Ala Glu Glu Leu	
530 535 540	
aag gaa atc agg ctg agg ccc tgg gag gcc cgc gtg tac aag atc agg	1680
Lys Glu Ile Arg Leu Arg Pro Trp Glu Ala Arg Val Tyr Lys Ile Arg	
545 550 555 560	
ctg cca tga	1689
Leu Pro	

<210> SEQ ID NO 58

<211> LENGTH: 562

<212> TYPE: PRT

<213> ORGANISM: unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 58

Met Glu Arg Val Trp Trp Lys Glu Ala Val Val Tyr Gln Ile Tyr Pro	
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20 25 30	
Ile Ile Ala Lys Leu Asp Tyr Leu Lys Glu Leu Gly Val Asp Val Val	
35 40 45	
Trp Leu Ser Pro Val Tyr Lys Ser Pro Asn Asp Asp Asn Gly Tyr Asp	
50 55 60	
Ile Ser Asp Tyr Arg Asp Ile Met Asp Glu Phe Gly Thr Met Ala Asp	
65 70 75 80	
Trp Lys Thr Met Leu Glu Glu Met His Lys Arg Gly Ile Lys Leu Val	
85 90 95	
Met Asp Leu Val Val Asn His Thr Ser Asp Glu His Pro Trp Phe Ile	
100 105 110	
Glu Ser Arg Lys Ser Lys Asp Asn Pro Tyr Arg Asp Tyr Tyr Ile Trp	
115 120 125	
Arg Pro Gly Lys Asn Gly Lys Glu Pro Asn Asn Trp Glu Ser Val Phe	
130 135 140	
Ser Gly Ser Ala Trp Glu Tyr Asp Glu Met Thr Gly Glu Tyr Tyr Leu	
145 150 155 160	
His Leu Phe Ser Lys Lys Gln Pro Asp Leu Asn Trp Glu Asn Pro Lys	
165 170 175	
Val Arg Arg Glu Val Tyr Glu Met Met Lys Phe Trp Leu Asp Lys Gly	
180 185 190	

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Val Asp Gly Phe Arg Met Asp	Val Ile Asn Met Ile Ser Lys Val Pro
195	200 205
Glu Leu Pro Asp Gly Glu Pro Gln Ser Gly Lys Lys Tyr Ala Ser Gly	
210	215 220
Ser Arg Tyr Tyr Met Asn Gly Pro Arg Val His Glu Phe Leu Gln Glu	
225	230 235 240
Met Asn Arg Glu Val Leu Ser Lys Tyr Asp Ile Met Thr Val Gly Glu	
	245 250 255
Thr Pro Gly Val Thr Pro Lys Glu Gly Ile Leu Tyr Thr Asp Pro Ser	
	260 265 270
Arg Arg Glu Leu Asn Met Val Phe Gln Phe Glu His Met Asp Leu Asp	
	275 280 285
Ser Gly Pro Gly Gly Lys Trp Asp Ile Arg Pro Trp Ser Leu Ala Asp	
	290 295 300
Leu Lys Lys Thr Met Thr Lys Trp Gln Lys Glu Leu Glu Gly Lys Gly	
305	310 315 320
Trp Asn Ser Leu Tyr Leu Asn Asn His Asp Gln Pro Arg Ala Val Ser	
	325 330 335
Arg Phe Gly Asp Asp Gly Lys Tyr Arg Val Glu Ser Ala Lys Met Leu	
	340 345 350
Ala Thr Phe Leu His Met Met Gln Gly Thr Pro Tyr Ile Tyr Gln Gly	
	355 360 365
Glu Glu Ile Gly Met Thr Asn Val Arg Phe Pro Ser Ile Glu Asp Tyr	
	370 375 380
Arg Asp Ile Glu Thr Leu Asn Met Tyr Lys Glu Arg Val Glu Glu Tyr	
385	390 395 400
Gly Glu Asp Pro Gln Glu Val Met Glu Lys Ile Tyr Tyr Lys Gly Arg	
	405 410 415
Asp Asn Ala Arg Thr Pro Met Gln Trp Asp Asp Ser Glu Asn Ala Gly	
	420 425 430
Phe Thr Ala Gly Thr Pro Trp Ile Pro Val Asn Pro Asn Tyr Lys Glu	
	435 440 445
Ile Asn Val Lys Ala Ala Leu Glu Asp Pro Asn Ser Val Phe His Tyr	
450	455 460
Tyr Lys Lys Leu Ile Gln Leu Arg Lys Gln His Asp Ile Ile Val Tyr	
465	470 475 480
Gly Thr Tyr Asp Leu Ile Leu Glu Asp Asp Pro Tyr Ile Tyr Arg Tyr	
	485 490 495
Thr Arg Thr Leu Gly Asn Glu Gln Leu Ile Val Ile Thr Asn Phe Ser	
	500 505 510
Glu Lys Thr Pro Val Phe Arg Leu Pro Asp His Ile Ile Tyr Lys Thr	
	515 520 525
Lys Glu Leu Leu Ile Ser Asn Tyr Asp Val Asp Glu Ala Glu Glu Leu	
	530 535 540
Lys Glu Ile Arg Leu Arg Pro Trp Glu Ala Arg Val Tyr Lys Ile Arg	
545	550 555 560
Leu Pro	

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That which is claimed:

1. A method comprising the steps of:

a) providing transgenic plant material comprising one or more locked carbohydrates and one or more key enzymes, wherein the one or more key enzymes is targeted away from the one or more locked carbohydrates; and

b) processing said transgenic plant material under conditions sufficient for one or more key enzymes to convert one or more locked carbohydrates to fermentable sugar.

2. The method of claim 1, wherein the one or more key enzymes is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

3. The method of claim 1, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

4. The method of claim 1, wherein the one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, and alpha-1,6-glucosidase.

5. The method of claim 1, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

6. A method comprising the steps of:

a) providing transgenic plant material comprising one or more lock enzymes, one or more locked carbohydrates and one or more key enzymes, wherein the one or more key enzymes is targeted away from the one or more locked carbohydrates; and

b) processing said transgenic plant material under conditions sufficient for said one or more key enzymes to convert said one or more locked carbohydrates to fermentable sugar.

7. The method of claim 6, wherein the one or more lock enzymes is selected from the group consisting of dextranase, levan sucrase, alternansucrase, sucrose isomerase and amylosucrase.

8. The method of claim 6, wherein the one or more key enzymes is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

9. The method of claim 6, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltulose, turanose and isomaltose.

10. The method of claim 6, wherein the one or more key enzymes is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, and alpha-1,6-glucosidase.

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11. The method of claim 6, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

12. A transgenic plant comprising one or more heterologous lock enzymes, one or more locked carbohydrates and one or more heterologous key enzymes, wherein the one or more key enzymes is targeted away from the locked carbohydrate.

13. The transgenic plant of claim 12, wherein the one or more lock enzymes is selected from the group consisting of dextranase, levan sucrase, alternansucrase, sucrose isomerase and amylosucrase.

14. The transgenic plant of claim 12, wherein the one or more key enzymes is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

15. The transgenic plant of claim 12, wherein the locked carbohydrate is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltose, turanose and isomaltose.

16. The transgenic plant of claim 12, wherein the one or more key enzyme is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, and alpha-1,6-glucosidase.

17. The transgenic plant of claim 12, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

18. A transgenic plant comprising one or more locked carbohydrates and one or more key enzymes, wherein the one or more key enzymes is targeted away from the one or more locked carbohydrates.

19. The transgenic plant of claim 18, wherein the key enzyme is targeted to an organelle selected from the group consisting of chloroplast, vacuole, cytoplasm, apoplast and endoplasmic reticulum.

20. The transgenic plant of claim 18, wherein the one or more locked carbohydrates is selected from the group consisting of isomaltulose, trehalulose, leucrose, starch, dextran, fructan, maltose, turanose and isomaltose.

21. The transgenic plant of claim 18, wherein the one or more key enzyme is selected from the group consisting of dextranase, alpha-amylase, glucoamylase, and alpha-1,6-glucosidase.

22. The transgenic plant of claim 18, wherein the transgenic plant is selected from the group consisting of maize, sugar beet, sorghum and sugarcane.

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